

*Mycelial development and phosphorus translocation
in systems of Phanerochaete velutina interacting
with resource restricted fungi*

*Thesis presented for the
Degree of Philosophiae Doctor
By*

Melanie Jane Harris B.Sc. (Hons)

*Cardiff School of Biosciences
University of Wales, Cardiff*

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Summary

The effect of arrival of wood resources, precolonised by either *Coriolus versicolor*, *Phlebia radiata*, *Stereum hirsutum* and *Vuilleminia comedens*, on mycelial systems of *Phanerochaete velutina* was studied in trays of non-sterile soil in the laboratory.

Morphological responses and nutrient movement were quantified non-destructively using respectively image analysis and ^{32}P monitoring with a scintillation probe, and subsequently destructively harvesting. The presence of a fungus occupying the new resource or state of decay of the resource arriving on an established system had major effects on deployment of biomass and on the uptake and allocation of phosphorus, the effects were specific to the species occupying the new resource. When new resources were added of constant size and decay state, massive redeployment of biomass away from regions with no new resource only occurred with three types of new resource: (1) uncolonised; (2) colonised by *V. comedens*; and (3) to a lesser extent those colonised by *S. hirsutum*. ^{32}P was taken up by *P. velutina* both in the vicinity of the inoculum and the new resource, and was translocated to the new resource from both sites of uptake, however the local supply contributed most. Bidirectional translocation also occurred. Initial ^{32}P translocation to wood resources precolonised for three years by *V.comedens* and *S.hirsutum* was initially high, greater initial translocation of ^{32}P occurred to younger precolonised resources of 8 weeks where a dramatic increase in P activity was apparent in systems containing the precoloniser *V.comedens*. Mycelial systems containing two resources, precolonised and/or uncolonised and at different states of decay had major effects on phosphorus translocation to these resources. Mycelial systems growing from different inoculum sizes effected the outcome of interactions between *P. velutina* and resource restricted fungi. Phosphorus translocation was also effected by inoculum to resource ratio within the system. Lower ^{32}P activity was observed in mycelial systems extending from 1cm^3 inoculum when compared to systems extending from 4cm^3 and 16cm^3 inoculum. An increase in inoculum to resource ratio increased the capability of cord systems of *P. velutina* to capture resources that were once occupied by other wood decay species.

These responses are discussed in relation to mycelial foraging strategies, nutrient translocation and partitioning within mycelial cord systems.

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Abbreviations and Terminology

ANOVA	Analysis of variance
CPM	Counts per minute
CPS	Counts per second
D _{BM}	Mass fractal dimension – measurement of space of whole structure
D _{BS}	Border fractal dimension – sum of the perimeters of the boundary of the structure
MA	Malt Agar
MINITAB ®	Statistical Software
Resource Units	Wood resource units / baits supplied to mycelium
SEM	Standard error of the mean
Semper ®	Image Analysis Software
R1	Wood resource 1
R2	Wood resource 2

CHAPTER 1

Introduction

1.1 Plant Fungal Associations

Fungi play an important role in terrestrial ecosystems as plant and animal pathogens, in mutualistic relationships with plants (mycorrhizas and lichens) and as saprotrophs recycling dead organic matter. Saprotrophic fungi are crucial agents of recycling Carbon and nutrients locked up in dead plant material in all terrestrial ecosystems not least forests. Though input of woody material to forest ecosystems is less than for other litter components (e.g. leaves and fruits), because of its recalcitrant nature, it forms a dominant component of litter on the forest floor, ranging between $x\text{-}y\text{ kg ha}^{-1}$ (Rayner & Boddy, 1988). Although nutrient concentrations in wood are less than in other litter components, the large standing crop of woody biomass on the forest floor represents a large nutrient investment by the ecosystem (Rayner & Boddy, 1988). Since basidiomycete and xylariaceae ascomycete fungi are major agents of wood decomposition, they must play a major role in the nutrient cycling of forests.

Though some basidiomycetes are capable of utilising all components of wood, it is very rare for a single fungus to be responsible for complete decomposition of a unit of wood in nature. Usually several or even many species of fungi are found in a single piece of wood, and the community changes with time (Rayner & Boddy, 1988). Fungi that cause wood decay (section 1.2) have been divided based on whether they are resource unit restricted or non-restricted. Unit restricted fungi are confined within their substrata and must rely on spore dispersal to reach new organic resources. Non

unit restricted, however, can egress from a resource via exploratory mycelial networks, often aggregated to form rhizomorphs or cords (section 1.3-1.5) and locate new plant litter resources as they grow out across the woodland floor. They are able to translocate nutrients (section 1.6) to the new resources to aid in colonisation. Different species and the same species under different conditions allocate mycelial biomass differently when searching for new resources (section 1.7). When new resources are encountered they reallocate biomass (section 1.7) and reallocate mineral nutrients (section 1.6). When new resources are encountered they will usually already be colonised, either by other cord-forming species or by unit restricted fungi. Aggressive interactions occurs which may or may not enable the newly invading cord-forming fungus to colonise the wood (section 1.8).

This thesis explores the effects on non unit-restricted fungi encountering non-colonised resources by unit-restricted fungi in terms of changes in mycelial pattern formation and translocation of phosphorus.

1.2 Decay of Wood Litter

Wood decay fungi are grouped according to their ability to break down the wall layers of woody cells. Five cell wall layers have been identified: the middle lamella and the primary wall composed largely of pectin and lignin; and three secondary walls that contain mostly cellulose (94%) and hemicellulose. Following death of a woody resource, cells are rapidly exhausted of low molecular weight compounds leaving the relatively recalcitrant molecules lignin, cellulose and hemicellulose. However, trees vary in their anatomical structure and precise composition of cell wall constituents.

Within the same individual and species and between species, diverse patterns of decay are observed. These can be partially attributed to the differing attractiveness of these cell wall layers to different fungal enzymes, but fluctuations in environmental conditions within the woody resource will also have substantial influence. Fungi are often distinguished on the basis of three types of wood decay, these being white, brown and soft rot (Rayner & Boddy, 1988) White rot fungi, occurring largely in basidiomycetes and ascomycetes, effect breakdown of lignin, cellulose and hemicellulose and cause wood to have a bleached appearance. White rot can be further divided into selective delignification and simultaneous rot. Selective delignification occurs in both broad leaved and coniferous trees. Lignin and hemicellulose is preferentially degraded first and only later is cellulose broken down. Fungi that cause simultaneous rot (in broad leaved trees only), degrade lignin, cellulose and hemicellulose at approximately the same rates. Brown rot is commonly caused by basidiomycetes (usually those belonging to the *Polyporaceae*), principally occurs in coniferous trees and is characterised by a powdery brown and cracked appearance. Cellulose and hemicellulose are broken down in brown rot but lignin remains albeit in a modified form.

Except following disturbance events, such as storm damage or felling, the decomposition process begins before wood litter is released from the canopy. Microbial decay may arise following wounding or via discontinuities in the bark. Heart rot fungi, causing top and butt rot are common occupants causing primary decay. In addition, decay may already be effected by latently present fungi. Dormancy of such latent invaders is broken when water saturated conditions of the living tree

have abated, following wounding, drought, root damage or fall of the tree (Boddy, 2001).

Fungi (mainly Basidiomycetes and to a lesser degree Pyrenomycete Ascomycetes) are the major agents of wood decomposition in terrestrial ecosystems (Rayner & Boddy, 1988). However, some bacteria, and imperfect fungi can be significant contributors during exposure to long term wet conditions, are also able to utilise cellulose (and even lignin) and can also deplete storage compounds found in ray cells. Invertebrates have an indirect effect on decomposition, since grazing and communitation by representatives of the Isoptera and Coleptera have been shown to alter the decomposer flora and release minerals. Direct decay of wood has also been demonstrated in termites though this is via internal and external microbial symbionts (Rayner & Boddy, 1998; Boddy & Watkinson, 1994).

1.3 Location of Mycelial Cords

Terrestrial ecosystem functioning depends on decomposition and redistribution of organic carbon and nutrients (Boddy & Watkinson, 1995). Saprotrophic cord forming fungi play an essential role in these processes, forming important conduits in the food chain and routing minerals back into primary productivity. They are prevalent on the forest floor of natural, tropical, temperate and boreal woodlands, and forests. They can also form nets which catch leaves in the canopy of tropical rain forests (Hedger, 1990). Cord forming fungi are usually Basidiomycetes, and some saprotrophically decompose plant litter (the subject of this thesis), though others are ectomycorrhizal biotrophs (e.g. *Suillus bovinus*) or weak pathogens (e.g. *Resinicium bicolor*). Cord

forming fungi are not restricted to woodland ecosystems, however, and function in sand dunes, dung, compost heaps, grasslands and in thatch (Boddy, 1993).

Non-unit restricted decomposer fungi grow across woodland floor, at or just below the soil/litter interface and interconnect spatially separated plant litter components. In such environments, aggregations of hyphae produce persistent and long lived linear organs able to extend over considerable distances and attain great size and age. Mycelial individuals of the cord former *Phanerochaete velutina* have been found to produce cords up to 5mm in diameter and 30 metres in length have been excavated (Thompson & Rayner, 1983). The indeterminate nature of cord systems mean that they may grow over infinitely large areas. Further, the rhizomorphic systems of *Armillaria bulbosa*, have been found to be among the largest and most persistent organisms on earth (Smith et al., 1992). Such aggregations may be an adaptation to desiccating conditions that may occur at the soil/litter interface (Thompson, 1984).

The precise form of exploratory outgrowth will depend on resource preference (Boddy, 1999). Fungi that forage for organic material that is homogeneously available will tend to form diffuse systems (Rayner & Boddy, 1988). Such foraging behaviour is typical of fungi colonising decomposing woodland and grassland litter (Dowson et al., 1989). Whereas those that interconnect spatially and temporally discrete units, will vary in foraging behaviour and hyphal aggregation of their linear organs according to environmental condition, species or ecological strategy.

There has been some confusion over description of exploratory mycelia (Boddy, 1999). In this study, linear aggregated organs formed behind a foraging margin will be referred to as cords, whereas those forming fully autonomous, apically dominant structures termed rhizomorphs (Cairney et al., 1989).

1.4 Structure of Mycelial Cords

Aggregated linear organs vary in their degree of tissue differentiation and degree of apical dominance (Rayner et al., 1985). At one extreme are rhizomorphs which grow from the apex and are differentiated internally through openly branched systems in which individual hyphal apices grow with aggregation further back from the margin, to those that are only loose aggregations of hyphae (Townsend, 1954; Thompson & Rayner, 1982).

Cord forming fungi studied thus far, produce linear organs that consist of distinct tissues, usually comprising an outer cortical layer or 'rind' which often contains crystal encrusted hyphae of similar diameter (Rayner & Boddy, 1988; Townsend, 1954; Thompson & Rayner, 1982; Watkinson, 1984). The medullary zone is found within the rind and consists of a matrix of thin-walled hyphae, thick walled fibres and wide vessel hyphae (20µm) (Rayner et al., 1985; Cairney et al., 1992). Rhizomorphic fungi such as *Armillaria spp.* are highly aggregated and within a 1mm diameter rhizomorph 1000 aggregations of hyphae may be entwined. Ahead of the elongation zone, the apical growing region consists of small isodimetric cells which are protected by an outer slimy matrix composed of intertwined hyphae (Webster, 1980).

1.5 Function and Mechanism of Nutrient Translocation in Mycelial Cords

Growth between spatially separated resource units across the forest floor is expensive in terms of carbon and nutrient cost. Cord forming fungi make efficient use of available nutrients by forming persistent systems that can utilise woody resources landing on them and by efficient foraging strategies (section 1.6 & 1.7). They redistribute biomass and nutrients to where demand is greatest, by means of translocation. Translocation as defined in the Oxford dictionary is 'to move (substances in the plant) from one part to another' In this study, translocation describes the situation where substances actually move between or within existing structures and excludes displacement of substances by growth.

By their very nature large mycelial systems growing under nutrient depauperate conditions (e.g *Armillaria spp* and *Serpula lacrimans*) were thought to be routes for mineral transfer (Butler, 1957; Ellis, 1929; Garret, 1954, 1956 and Webster, 1980). However until the development of tracer studies evidence for translocation was only circumstantial. The movement of substances labelled with dyes or radiolabelled nutrients provided qualitative evidence that translocation could occur (Lucas, 1977).

It is clear that saprotrophic Basidiomycete cord systems are the functional pathways for the translocation of nutrients between distant resources (Wells & Boddy, 1990). Nutrient uptake and subsequent allocation by wood decay and mycorrhizal fungi have been quantified using radiolabelled phosphorus (as ^{32}P) (Wells & Boddy, 1990; Wells et al, 1990; Hughes & Boddy, 1994; Hughes & Boddy, 1996), nitrogen ^{15}N (Finlay et al., 1988) and carbon, ^{14}C (Wells et al., 1995, Wells et al., 2002).

The actual mechanism of translocation is not clear, though the route of translocation over large distances is thought to be the wide vessel hyphae (Cairney, 1992; Cairney et al., 1989). This is based on circumstantial evidence that calculated fluxes through wide vessel are similar to observed fluxes. It is possible, however, for translocation to occur in different vessels and for translocation to occur in opposite directions at the same time. Translocation of radiolabelled phosphorus in a rhizomorphic ectomycorrhizal cord former, was found to be associated with younger and cortical hyphae with cytoplasmic contents, rather than wide vessel hyphae (Duddridge et al., 1988).

Previous, qualitative studies have shown that reallocation of mycelial biomass from non-connective to newly encountered food resources connected to a bait can occur (Dowson et al., 1988a, 1988b, 1998c, 1989). Foraging strategies vary with species, state of decay, size and type of inoculum and new resources (Dowson et al., 1988a, 1988b, 1988c, 1989). Using radiolabelled phosphorus (as ^{32}P), subsequent studies have sought to investigate nutrient uptake, translocation and reallocation in relation to these observed foraging strategies (Wells et al., 1990).

To determine phosphorus pathways in mycelial systems, radiolabelled orthophosphate has been used (Beever & Burns, 1980, Wells, 1990). Such studies typically measure the uptake, translocation and reallocation of nutrients between inocula, connective/non-connective mycelium and fresh wood resources (baits) added variously in time and space and under differing abiotic/biotic conditions (Boddy, 1999). Using scintillation counting, early studies quantified phosphorus activity (as ^{32}P) destructively in model laboratory based soil systems (harvesting mycelia, baits

and inocula) (Wells, 1990). Phosphorus activity can also be quantified (around and within wood resources) non-destructively using a scintillation probe attached to a rate meter, allowing for precise measurement in space and over time (Hughes, 1993; Hughes & Boddy, 1994; Wells, 1990; Wells & Boddy, 1995a). In these studies, however, phosphorus (as ^{32}P orthophosphate) was only added to specific compartments, setting up a phosphorus concentration gradient across the system. Subsequently, gradient free systems were developed (Wells, et al., 1997, 1998a, 1998b, 2000), where phosphorus was applied evenly across the soil model systems at 'realistic levels' (as ^{32}P orthophosphate added to the radiolabelled compartment and 20mM $\text{NH}_4\text{K}_2\text{PO}_4$ added to the remaining area of the system). Translocation studies using radiolabelled orthophosphate have also been undertaken in the field (Clipson, Cairney & Jennings, 1987; Wells & Boddy, 1995b). In these early studies, the distribution of phosphorus activity between litter components was highly variable and the complex relationships were difficult to interpret (Boddy, 1993, Hughes & Boddy, 1994). Wells & Boddy (1995b) showed movement of ^{32}P from a central inoculum to organic resources at the edges of system over distances up to 75cm.

As well as monitoring nutrient pathways through mycelial networks, other studies have also been undertaken to further understand the dynamics of the transport system through foraging mycelial systems of *P. velutina*. Photon counting scintillation imaging (PCSI), a technique employed to determine amino acid translocation in mycelial networks, non-destructively, where radiolabelled compounds are imaged continuously with time (Tlalka, et al., 2002; Tlalka et al., 2003).

A number of biotic and abiotic factors can influence phosphorus translocation through mycelial cord systems. Studies have shown that allocation and flux rates of ^{32}P are dependent on the decay state of the food resource base and of baits (Wells & Boddy 1990; Hughes, 1993). Flux rates as high as $7225 \text{ nmol P cm}^{-2} \text{ d}^{-1}$ and $4214 \text{ nmol P cm}^{-2} \text{ d}^{-1}$ have been reported from 39 week old inocula, through connective cords towards spatially separated fresh baits of *Phanerochaete velutina* and *Phallus impudicus* respectively (Wells et al., 1990). Increased decay of the inocula, resulted in an increase in the proportion of phosphorus activity allocated to newly encountered baits and connective mycelium at the expense of non-connective mycelium and the resource base. Only a small proportion – typically less than 20% - of the total allocated nutrients was located in extra resource mycelium indicating that nutrients are stored in ‘safe’ woody resources and, consequently, death of mycelial systems will return relatively small amounts of nutrients to the soil (Wells et al, 1990; Hughes, 1993; Wells & Boddy, 1995a; Wells et al., 2000). It has been suggested that uptake of phosphorus from soil is ‘opportunistic’ (Wells et al., 1990), but later work suggested uptake to increase following sequential encounter of wood baits (Hughes & Boddy, 1996) and, under carbon limiting conditions, energy may be directed towards foraging for new resources rather than nutrient acquisition from the soil (Wells & Boddy, 1995a). Furthermore, when mycelia of *Phanerochaete velutina* grew on nutrient depleted soil and wood resources subsequently added, differentiated patches of diffuse hyphae developed (Wells et al., 1997). These patches were thought to be signs of increased nutrient uptake, since phosphorus uptake was 3 times higher when radiolabelled ^{32}P was added to patches rather than existing cords, and patch formation ceased following fertiliser treatment (Wells et al., 1998).

The direction of translocation in model soil systems appears to be influenced by resource unit availability (Wells et al, 1990, 1998). Acropetal translocation was favoured by *Phanerochaete velutina* and *Phallus impudicus* foraging from inocula bases towards new baits, whilst basipetal movement was observed towards inoculum bases from distal exploratory mycelial cords (Wells et al, 1990). Such findings indicate that phosphorus flux may be controlled by source-sink relationships and that scavenged nutrients (phosphorus) and possibly mycelial biomass (Dowson et al., 1986) are partitioned according to the carbon content of potential resources, their size (Wells et al, 1990; Hughes, 1993; Hughes & Boddy, 1996) and the number of, and time at which baits are added (Hughes, 1993; Hughes & Boddy, 1996). When encountering a sequence of new resources differing in size, more phosphorus was always allocated to larger resources, irrespective of where in the sequence it was encountered (Hughes, 1993; Hughes & Boddy, 1996); and demand for phosphorus is highest at early stages of colonisation (Hughes & Boddy, 1993).

In later studies, however, Wells et al., (1998,1998b,1999), questioned the hypothesis that the pathway of translocation was demand driven (Cairney 1992). Acquisition rates of phosphorus translocated from inocula to multiple baits added pairwise over a 60d period was not greatest in the most recently added baits. Initially older resources were the main sinks for translocated phosphorus, but acquisition rates to most recently added baits increased with time and was supported by efflux from older baits (Wells et al, 1998a). In a sister study, inoculum size did not affect total phosphorus acquisition by wood resources, but phosphorus allocated to resources, that had been in contact with mycelium for different times was affected by inoculum size (Wells et al., 1999). Such studies provided evidence that translocation may be partitioned according

to where it is being stored and actively used (Boddy, 1999) rather than a process driven by metabolic demand (Cairney, 1992).

Resource type has also been identified as a possible factor influencing phosphorus partitioning in mycelial systems (Wells et al., 1995; Wells & Boddy, 1995a). In a field study, Wells & Boddy (1995b) found that allocation of phosphorus (as ^{32}P) to plant litter resources (over distances of up to 75cm) varied between species. Of particular interest was the finding that the growing apices of mosses can rapidly gain phosphorus from fungal mycelium. *Hypholoma fasciculare* frequently translocated phosphorus to colonised living/dead roots and leaf litter whereas *Phanerochaete velutina* or *Phallus impudicus* did so rarely. In the laboratory (Wells et al, 1990) it has been shown that *Phanerochaete velutina* caused greater decay and allocated greater amounts of phosphorus to standard wood baits than leaf litter. However, this may not completely reflect a preference, but merely suggest that leaf litter contains greater amounts of phosphorus and reallocation to be dependent on availability of phosphorus in different parts of the system (Wells, et al., 1990).

Microcosm studies investigating the affects of abiotic factors such as nutrient supply (Wells, et al., 1998), nutrient availability (Wells, et al., 1997), soil water potential shifts (Wells, et al., 2000) and temperature (Wells, et al., 1995c) on phosphorus translocation and mycelial development (Dowson et al, 1989a; Donnelly & Boddy, 1997b; Wells & Boddy, 1995c) have also been undertaken, though these have been under constant conditions. Such work fails to take in to account dynamic fluctuations in environmental factors such as resource moisture availability and temperature that occur under natural conditions (Boddy, 1984). However, attempts have been made to

mimic more 'realistic' shifts in temperature (Wells et al., 1990) and water availability (Wells et al., 2000) and to monitor their effects on phosphorus partitioning and uptake. The temperature at which cords develop (10°C and 25°C) and the temperature during uptake (5°C – 25°C) was shown to affect translocation and uptake of phosphorus. The rate of uptake and translocation was found to be species and temperature dependent. Acclimation to temperature was demonstrated in *Phanerochaete velutina* and *Hypholoma fasciculare*, since the initial translocation rates when initially grown at 10°C were higher at all temperatures and total phosphorus translocation were higher at lower temperatures than when grown initially at 25°C. (Wells et al., 1995c). Further, *Phanerochaete velutina* had a lower optimal temperature for phosphorus uptake and translocation. This adaptation has clear advantages for phosphorus scavenging in environments where temperatures are commonly at or below 15°C, and rise above this in spring and summer (Wells et al., 1995c).

Temporal shifts in temperature have also been shown to affect phosphorus partitioning (Boddy, 1999). Shifting temperature from 12.5°C to either 5°C or 20°C (at the time of addition of a fresh bait), resulted in a greater proportion of phosphorus allocated to fresh baits added to mature systems behind the mycelial front, indicating that the fresh resource aided as a larger nutrient sink than other resources in the system (Boddy, 1999). Further, reducing the temperature from 12.5°C to 5°C, reduced the ability of the mycelium to scavenge nutrients local to the newly acquired resource. Wells et al. (2000), in another complex microcosm study, shifted water availability (from 0.019 MPa to either 0.009MPa or 0.056 MPa) either at the same time or 20d following encounter of fresh wood resources, and found that phosphorous

partitioning and uptake depended on both the time and direction of soil matric potential change. Though no significant difference ($P < 0.05$) in total phosphorus uptake could be found between wet shift (water potential change same time as the addition of fresh wood bait) and control systems, delaying change in soil matric potential by 20d resulted in significantly greater phosphorus uptake (mean of 65% increase over controls). In contrast, total phosphorus uptake was significantly reduced ($P < 0.05$) when soil matric potential was reduced immediately after the supply of baits or after 20d following the same (Wells et al., 2000). In addition, dry shifting (following delayed and immediate supply of baits) reduced the proportion of phosphorus translocated to inocula, though only significantly ($P < 0.05$) when water potential was reduced at the time of encounter of fresh baits.

These studies provide clues to how spatially and temporally changing environmental conditions affect foraging patterns, nutrient acquisition and reallocation (Wells & Boddy, 1995a; Wells, et al., 1997b, 1998, 1998b, 1999; Hughes, 1993; Hughes & Boddy, 1996). In order that the internal routes and mechanisms of translocation are properly understood, future studies must be designed such that soil tray microcosms better mimic the heterogeneous environment that cord forming fungi are subject to (Boddy, 1999).

1.6 Foraging Strategies of Mycelial Cords – Qualitative Data

During outgrowth from woody food bases, difference in morphology of exploratory cord systems has been attributed to different foraging strategies (Dowson et al., 1988a). Laboratory experimentation using model soil tray systems have investigated foraging patterns of many cord forming species, though *Phanerochaete velutina*,

Hypholoma fasciculare and *Phallus impudicus* are most commonly used as model species (Abdalla & Boddy, 1996; Dowson et al., 1986,1989; Hughes, 1993; Hughes & Boddy, 1994; Wells et al., 1990; Wells & Boddy 1990; Wells & Boddy 1995b; Owens S, 1997). These experiments have been conducted in bioassay trays in which mycelium egresses from colonised wood resources across non-sterile compacted soil towards uncolonised baits. Qualitative studies in the laboratory (Dowson et al., 1986, 1988a, 1989) and in the field (Dowson et al., 1988b, 1988c; Wells et al., 1995b) have revealed that cord systems initially grow out from food resources respond to local micro-environmental conditions (in the absence of a bait), but once a new resource has been encountered considerable reallocation of mycelial biomass occurs (Wells et al., 1990). Thickening of cords connected to a new bait and regression of mycelium not connected to the same is typically observed (Dowson, et al., 1988a, 1988b, 1988c, 1989). This results in polarised, directed growth towards new food resources and away from previously colonised baits and inocula (Dowson et al., 1988b, Hughes, 1993). The extent of regression depends on species and on the ratio of inoculum : bait. Differences have also been found depending on the soil: in a study investigating outgrowth patterns on angiospermous or coniferous litter, soil and humus, Abdalla & Boddy (1996) report that regression of *Phanerochaete velutina* non-connective mycelium to occur only occasionally in coniferous litter and thickening to occur only on angiospermous litter, soil or humus soil systems.

Dowson et al (1986, 1988a) suggested that foraging patterns could be divided into 'long' (Guerilla) and 'short range' (Phalanx) strategies, analogous to those of plants and animals (Schmid & Harper 1985) Its has been suggested that short range strategies are employed by cord forming fungi that typically exhibit slow, densely

branched growth patterns. Such adaptations indicate a strategy for short range, unspecialised, combative foraging that maximises resource capture over short distances. Resources that are distributed relatively homogeneously often induce diffuse exploratory mycelia. Scavengers of woodland litter, *Clitocybe* spp., *Collybia* spp. and *Mycena* spp., and grasslands, *Marasmius oreades* adopt such a foraging strategy (Boddy, 1999). Cord forming species such as *Hypholoma fasciculare* and *Steccherinum fimbriatum* also exhibit this growth pattern. Longer range strategies are operated by cord forming fungi such as *Phanerochaete velutina*, *Phanerochaete laevis* and *Phallus impudicus*. The former two species are highly combative and are able to rapidly colonise widely separated woody resources from sparse extra resource mycelia. *Phallus impudicus*, in contrast, is less combative and causes less decay, but produces more persistent, rhizomorphic cords enhancing the chances of successful resource capture when food resources are encountered (Dowson et al., 1988).

Following these early qualitative studies, much research has been undertaken to quantify the observed shifts in outgrowth patterns and to investigate how factors such as resource type, quality, quantity the presence of antagonists or the microenvironment affect foraging strategies (Boddy, 1999) (section 1.7).

1.7 Quantification of Mycelial Patterns Using Fractal Geometry

The development of image processing technology has permitted the objective measurement of microbial (Obert et al., 1989) and later mycelial growth of different species growing in different environments (Bolton et al, 1991; Bolton & Boddy, 1993; Donnelly et al, 1995, 1997, 1998). Alongside visual observations, non-destructive

determination of biomass, hyphal cover and radial extension can be made using this image analysis technology. In recent studies fractal dimension, together with the aforementioned descriptors has also been used to describe and define mycelial systems in model laboratory soil systems (Boddy, 1999; Donnelly et al., 1995).

Many natural irregular shapes for example; mycelia, are approximately fractal. Two-dimensional structures such as linear mycelial systems growing over a thin layer of soil in the laboratory, have a fractal dimension between 1 and 2; 1 being a line and 2 being a filled plane. Two fractal dimensions can be calculated for the digital images of mycelial systems.

As mycelial cord systems are formed behind a growing margin, it is useful to be able to quantify the degree of space filling within the whole system and at search fronts. D_{BS} (surface / border fractal) describes systems that are only fractal (plane filled) at their boundaries and D_{BM} (mass fractal) describes systems where there are gaps in the interior.

The development of the box counting method (Donnelly et al., 1995) allowed for measurement of these two fractal dimensions, and the degree to which a structure is mass fractal (D_{BM}) or surface fractal (D_{BS}) can be determined by calculating the ratio of D_{BS}/D_{BM} . Values approaching 1 indicate a mass fractal structure and lower values indicate structures that have plane filling regions (Donnelly et al., 1995). Mass fractal dimensions (D_{BM}) are indicative of the foraging strategy exhibited by a mycelial system at a given time. High values of D_{BM} indicate, a highly branched system and a short range 'Phalanx' strategy, whereas systems with lower values of D_{BM} will be

more open and are indicative of long range 'guerrilla' strategists (Boddy, 1999; Owens, 1997).

Using image analysis and fractal geometry to describe mycelial systems in soil microcosms, Donnelly, et al., (1995) confirmed the earlier distinction between the two broad growth forms, and that they vary within individuals and between species according to environmental conditions, resource type and size (Abdalla & Boddy, 1996; Donnelly et al., 1997). Those which are mass fractal, and have rapidly extending openly branched cords, e.g. *Phanerochaete velutina*, *Phallus impudicus* and *Agrocybe gibberosa* and systems where D_{BS} is not equal to D_{BM} , were termed surface fractal and characterised by slow-diffuse growth exhibited by *Hypholoma fasciculare*, *Megacollybia platyphylla*, *Stropharia aeruginosa* and *Stropharia caerulea*. However, these systems became more mass fractal. Fractal dimensions not only, change over time (Boddy, 1999; Donnelly et al., 1995) but also under conditions of altered nutrient status (Boddy, 1999; Donnelly & Boddy, 1997) water potential (Donnelly & Boddy, 1997; Dowson et al., 1989; Wells et al., 2000) and temperature (Donnelly & Boddy, 1997; Dowson et al., 1989; Wells, et al., 1995c), though these studies have concentrated on a relatively small number of species

1.7.1 Change in mycelial pattern formation in relation to different abiotic conditions

Wells, et al. (1995c) found biomass and decay rates of *Phanerochaete velutina* to increase with temperature (5-26°C), reflecting higher metabolic activity at optimal temperatures. Mycelial extension rate of *Phallus impudicus* and *Hypholoma*

fasciculare increased with increasing temperature (5-25°C), though this was not always correlated with biomass, since diffuse growth was more evident at lower temperatures and more defined cords evident at lower temperatures; the converse true for *Phanerochaete velutina*. It has been suggested that poor correlation between growth parameters and decay rates maybe evidence that metabolic processes for intrasource and extrasource mycelium are separate (Donnelly et al., 1997). Sensitivity in extrasource mycelia to changes in the environment may allow for rapid developmental metabolic shifts that are more economic for carbon (Donnelly et al., 1997). *Stropharia caerula*, for example, was sensitive to temperature above 20°C, and this was reflected in low fractal values (D_{BM}/D_{BS}) at temperatures above 15°C and characterised by a switch between diffuse and aggregated cord growth with temperature increase. *Phanerochaete velutina* was less sensitive to such shifts in temperature.

Increases and decreases in soil water potential (from 0.019 MPa to either 0.056 MPa or 0.009 MP) caused polarised growth of *Phanerochaete velutina*, increasing system space filling (D_{BM}) in baited over non baited sectors of soil trays, though regression of non-connective mycelium was more evident at 0.009 MPa than 0.056 MPa (Wells, 2000). Though species dependent, there was a tendency for extension rates, biomass and D_{BM} to decrease with decreasing water potential (Donnelly et al., 1997) and production of more aggregated cords in soils may be an adaptation to desiccating conditions (Dowson et al., 1989; Donnelly et al, 1997).

Under growth limiting conditions, cord-forming fungi are able to respond rapidly to changing environmental conditions that will occur in nature. Aggregation of cords and

more open branching behind a diffuse foraging margin and regression of non-connective mycelium may allow for more efficient redistribution of nutrients and biomass, providing mechanisms to maintain a functional exploratory mycelium until more favourable conditions are encountered.

1.8 Interactions between Mycelial Cords and Resource Restricted Fungi.

Phanerochaete velutina is a highly combative fungus (Dowson et al., 1988a) able to extend from wood across soil and successfully colonise territory in wood occupied by other fungi. Experiments in the field (Coates & Rayner, 1985 a,b,c; Chapela et al., 1988; Dowson et al., 1989; Wells et al., 1995) and in the laboratory (Boddy & Abdalla, 1998; Dowson et al., 1989; Holmer & Stenlid, 1993; Holmer, et al., 1997; Holmer & Stenlid, 1997) confirm that cord formers generally replace fungi found at earlier stages of the decomposition process. Success in the location and occupation of resources can be related to the ecological niche that cord forming fungi occupy, since the adaptations for secondary resource capture are in stark contrast to those necessary for primary resource capture of virgin resources (Boddy, 2000).

Since plant litter falling to the woodland floor is likely to be already colonised by active mycelia or latently present propagules, effective combat must follow encounter of new organic resources (Boddy, 1993). Success in combat depends on what fungi are already present and the combative ability of the potential invader (Boddy, 2001). Furthermore, acquisition of territory held by preceding species may be influenced by differential metabolism of cell wall components or whether substances inhibitory to the invader have been broken down. Laboratory studies demonstrated by Holmer &

Stenlid, (1993), suggest that predecessor / successor relationships may exist, since secondary colonisers generally replaced primary decayers found in natural combinations, even when the former inoculum size was 1/12 of the latter. The size of antagonists, the state of decay (Boddy & Abdalla, 1998) and input/output of fresh resources (Holmer & Stenlid, 1996) can also influence outcome of combative encounters. In a soil microcosm study *Phanerochaete velutina*, for example, replaced the resource unit restricted *Stereum hirsutum*, *Stereum rugosum* and *Ustilina deusta* in pairwise interactions, but replaced and colonised only the latter when they were confronted together. Furthermore, *Phanerochaete velutina* preferentially colonised uncolonised baits over baits colonised for 6 weeks which was in turn preferred to baits inoculated for 2 years. This could be attributed to a greater defensive ability exhibited by the precolonisers where the state of decay was greater in these resources when compared to resources previously uncolonised or precolonised by other wood decay fungi, or that the invader *P. velutina* favoured resources with greater available carbon content, i.e. previously uncolonised or precolonised 6 week old resources (Boddy & Abdalla, 1998).

Abiotic factors can affect combative ability of cord forming fungi (Boddy, 2000). Gaseous regime (Boddy et al., 1985), water potential (Griffith & Boddy, 1991), the presence of living callus tissue (Hendry, 1993) and temperature have all been shown to affect the outcome of combative encounters between wood decay fungi. Cord forming fungi, in particular, were found to be less combative under conditions of elevated carbon dioxide and reduced oxygen. Such studies emphasise the difficulty in predicting the composition of fungal communities, as the combative ability of

individual species will depend on their adaptations to constantly changing abiotic and biotic conditions found in nature (Boddy, 2000).

Caution must always be exercised when interpreting evidence from laboratory studies, as the interactions are usually between paired antagonists and performed on: agar, soil microcosms and using autoclaved wood blocks that were subsequently colonised for the same time for all species. Such treatments may affect fungi belonging to different ecological groups differently and, therefore, influence outcomes of interactions. However, useful eco-physiological information can be obtained from interaction studies under artificial conditions and be a stimulus to investigate new lines of enquiry. Pearce (1990), for example, found a close relationship between outcome of interactions between *Armillaria luteobubalina* and a range of antagonists on 3% malt agar plates and in wood lengths. Dowson et al. (1989), however, found that combative ability of cord-formers to be dependent on whether pairings were performed on agar, in wood lengths or in soil microcosms, but overall *Phanerochaete velutina* was found to be the most combative of the cord-formers tested. Furthermore, in resources colonised for different time (6 and 24 weeks) *Phanerochaete velutina* was found to be most combative when paired against *Hypholoma fasciculare* and *Phallus impudicus*. (Wells et al., 2002). However, since *Steccherinum fimbriatum* was more combative in wood lengths rather than soil, and reverse being true for *Phallus impudicus* (Wells et al., 2002) it is hypothesised that outcomes of interaction may vary according to developmental stage of contacting hyphae.

Aggregations of interacting hyphae are sites of non self fusion (Ainsworth & Rayner, 1986) and characterised by pigmentation, lytic responses and usually death of mycelia

behind the point of interference. At the point of contact between two (or more) opposing mycelial fronts, the developmental pathway hyphae are expressing may influence their combative ability in that region. Therefore, outcomes of interaction, may be dependent on where they take place (Wells et al., 2002).

Few studies have sought to investigate outcomes of interactions between unit restricted and non-unit restricted fungi, or how they affect nutrient acquisition and translocation, or mycelial outgrowth patterns and inocula/bait decay rates (Wells et al, 2002). Boddy & Abdalla, (1998), however, investigated mycelial outgrowth patterns and decay rates of *Phanerochaete velutina* (in soil microcosms) following contact with single colonised and uncolonised baits. Regression of non-connective and thickening of connective mycelia occurred when confronting *Stereum rugosum*, but not when encountering baits colonised by *Stereum hirsutum*, *Phlebia radiata*, *Coriolos versicolor*, *Xylaria polymorpha*, *Ustilina deusta* or uncolonised wood blocks, the latter contrary to earlier findings (Hughes, 1993). In microcosms, where *P. velutina* confronted more than one bait colonised by different species or an uncolonised block, regression of non-connective mycelium was not evident, but thickening of cords connected to the preferred bait was commonly observed. Time taken to egress from newly colonised baits was slower from precolonised baits than uncolonised, maybe reflecting the increased energy expenditure for acquiring territory. Whether in single or multiple baited systems, decay rates of baits were not commonly affected by decay state, size or species, though *P. velutina* inocula decay rate increased in the presence as opposed to absence of baits and when confronted with 8cm³ rather than 0.5cm³ wood baits; indicating an energy cost for resource capture (Boddy & Abdalla, 1998).

Nutrient exchange and decay partitioning have been found to occur during confrontations between the cord forming *Phanerochaete velutina*, *Hypholoma fasciculare* and *Phallus impudicus*. (Wells & Boddy et al., 2002). Leakage of ^{14}C (as glucose) by *Phanerochaete velutina* and *Hypholoma fasciculare* and uptake by *Phallus impudicus*, may indicate that there is a significant carbon cost during interspecific interactions, though this only occurred when antagonists were in deadlock. Furthermore, carbon loss (dry weight loss) was generally lower in self rather than non-self pairings, but during non-self-pairings initial carbon loss (^{14}C as glucose), depended on inoculum age and species. *Phanerochaete velutina* (24 week old inocula) and *Hypholoma fasciculare* (6 week and 24 week old inocula), for example, had greater initial carbon loss when paired against all other species / inoculum age combinations. In soil systems, opportunistically acquired carbon from an opponent's territory, however, did not correlate with outcomes of interaction, whereas those in soil did.

Nutrient exchange during interactions between different cord forming species (Wells et al., 2002) and between cord forming fungi and ectomycorrhizal fungi (Lindahl et al., 1999) has now been demonstrated, and may indicate a significant way minerals are routed back into primary production. Further experiments, under more natural conditions, may reveal the extent to which interspecific encounters contribute to the nutrient availability, partitioning and recycling in terrestrial ecosystems.

1.9 Aims and Objectives

The aim of this thesis was to further elucidate interactions between non-resource restricted fungi (in this case *P.velutina*) and resource unit restricted fungi having different ecological roles: *Vuilleminia comedens* (a primary coloniser of attached branches with poor combative ability), *Coriolus versicolor*, *Phlebia radiata* and *Stereum hirsutum* (secondary colonisers). In particular to simulate the arrival of colonised woody resources on an established system of *P.velutina*, and to determine *P.velutina* mycelial responses in terms of changes in fractal dimension and translocation of phosphorus from the *P.velutina* inoculum resource to foraging and colony fronts.

Changes in fractal dimension (chapter 3) and phosphorus translocation (chapter 4) were investigated in detail in mycelial systems to which woody resources colonised by the four aforementioned fungi were added, compared to systems to which uncolonised woody resources were added. Effect of the state of decay of precolonised newly added resources (chapter 5) and of different sizes of *P.velutina* inoculum (chapter 6) on outcome of interactions and phosphorus translocation was then examined.

CHAPTER 2

General Methods and Materials

2.1 *Fungal isolates*

The Cord forming basidiomycete *Phanerochaete velutina* was originally isolated from decaying beech (*Fagus sylvatica* L.) wood from the Forest of Dean, UK, (National Grid Reference SO 611145).

Four resource restricted basidiomycetes were used: *Coriolus versicolor*, *Stereum hirsutum*, *Vuilleminia comedens* and *Phlebia radiata*. Species were collected from the Forest of Dean, UK, (National Grid Reference SO 611145) and were isolated from decaying beech wood and from fruiting bodies. Fungal identification was made on fruiting body morphology and isolation material was aseptically transferred on to kanamycin agar plates and incubated at 20 °C in darkness. Mycelial growth was examined and once suitable growth occurred 5mm plugs were removed from the mycelial margin and were aseptically transferred onto MEA plates and incubated at 20°C in darkness. To maintain laboratory stocks of all isolates were routinely sub-cultured on 2% W/V malt extract agar (MA; 20g l⁻¹ Munton & Fison spray malt with 15g l⁻¹ Beta Lab agar).

2.2 *Growth Media*

For all isolations and fungal sub-culturing 90mm non-vented plastic Petri dishes were used. For general fungal sub-culture 2% malt extract agar (MA) was used and this was prepared by addition of 10g spraymalt A (Munton & Fisson, Stowmarket, UK) and 7.5g

Lab M agar No 2 (Amersham, Bury.UK) to 500ml of distilled water, and autoclaved at 121°C for 15 minutes. Kanomycin agar was prepared for the initial isolation from fruit body material by introducing 1ml of kanomycin antibiotic to 500ml sterile molten agar at 40°C. All plates were poured in a sterile class1 cabinet.

2.3 *Fungal Sub-culturing*

All species were routinely sub-cultured every 3-4 weeks by removing of 5mm diameter agar plugs using a sterile cork-borer (No. 3) from the margin of actively growing mycelia. The plugs were aseptically transferred and placed colony side down onto malt extract agar. Plates were incubated in darkness at 20°C

2.4 *Preparation of slope and flask cultures*

Five ml slopes of MA in 20ml glass universal bottles were each inoculated with colonised plugs of MA, and incubated at 20°C. Once sufficient mycelial growth was established bottles were transferred for storage in a 4°C incubator in darkness.

For the production of flask cultures, colonised agar plates were completely cut into 2 x 1cm² sections and all sections were aseptically transferred into wide-necked 2 Litre conical flasks containing 500ml of MA. Flasks were incubated for 2-3 weeks by which time the agar surface was completely colonised.

2.5 *Preparation of inocula wood blocks.*

Beech (*Fagus sylvatica*) trees were felled and cut into 50 x 10 x 2 cm³ planks by the Forest Authority and then depending on experimental design wood blocks sizes 20×20×10 mm³ or 10×10×10 mm³ were cut and were stored at –18°C in a chest freezer until required. from freshly felled beech. The appropriate numbers of wood blocks needed were defrosted, soaked overnight in deionised water to ensure all blocks were of the same moisture content. Wood blocks were sealed within two layers of aluminium foil and were then steam autoclaved twice for 30 minutes at 121°C at 24 hour intervals. The autoclaved wood blocks were transferred aseptically into wide-necked 2 Litre conical flasks containing 2-3 week old cultures of the test fungi grown on 500ml 2% MA. Blocks were placed in a uniform layer to ensure the same degree of colonisation. The flasks were then incubated at 20°C for 8 weeks (chapter 3, 4, 5, 6) and 3 years (chapter 5) to ensure full colonisation of the wood blocks.

2.6 *Preparation of uncolonised wood baits*

Wood blocks of known volume cut from a freshly felled beech tree were removed from the freezer. The required numbers of blocks were allowed to soak overnight in distilled water. The fresh weight and dimensions of the blocks were measured by vernier calipers and were recorded. The moisture content and relative density of the 6 wood block baits were estimated as described below.

2.7 *Determination of relative density and water content of wood inocula and baits*

Any surface mycelium or agar was scraped free from the wood blocks. They were then weighed, measured and oven dried at 80°C overnight. Wood blocks were re-weighed after cooling in a desiccator. The percentage moisture content and relative density of the inocula were calculated.

Relative density = Oven dry weight (g) / fresh volume (cm³)

Percentage Moisture content = (Fresh weight - Oven dry weight) / Oven dry weight) × 100

2.8 MODEL SOIL SYSTEMS

2.8.1 *Soil*

Non-sterile soil was used in experiments. Soil was collected from a mixed deciduous woodland (Tintern, UK; National Grid Reference SO 528018). Coarse leaf and wood litter was removed and the soil was sieved through a 10mm mesh before air-drying in the laboratory.

Soil water potential (ψ) was determined using the method of Fawcett & Collis-George (1967). A characteristic curve relating soil water potential to moisture content (% oven dry weight) of the soil used in all experimental studies was constructed. Soil, air-dried and sieved through a 2mm mesh, was wetted by adding different volumes of deionised water to give a range of moisture contents ranging from unsaturated to water saturated approximately field capacity. Glass dishes (9cm diameter) were half filled with each wet batch of soil and compressed in the base of the dish. Three 5cm diameter air-dried filter

papers (Whatman No.42) were placed on top of the compressed soil; the central filter paper being cut to a smaller size square to avoid contact with the soil. Soil was then added to the dishes and compressed. All dishes were sealed with Nesco Film to avoid water loss. Dishes were incubated in the dark for 14 d at 20°C. 4-5 replicates for each moisture content were set up. After 14 d the central filter paper for each sample was removed taking care to avoid contact with the soil. Fresh weight and oven dry weight (80°C) was determined for each filter paper. Soil moisture content was determined by oven drying the soil from each dish. Filter paper water potential can be estimated from a calibration curve – MPa v moisture content (Fawcett & Collis-George 1967). The moisture characteristic curve of the soil is then plotted by soil water content (OD.wt) v Water potential (ψ). In all experimental studies soil matrix potential of the bulk mixture was adjusted to -0.012MPa (Fawcett & Collis-George, 1967) by addition of deionised water.

2.8.2 Preparation of soil trays

For radiotracer studies, radioisotope compartments were needed, depending upon experimental design. For this purpose, Petri plates (9, 5 or 3.5cm diameters) were glued inside 24cm² Perspex trays (Fig 4.1, 5.1 and 6.1).

Soil was compressed evenly into the trays to the same depth of the Petri plates, approx. 5mm so that mycelial growth would be predominately on the surface of the soil. Prior to inoculation of the soil trays the soil was frozen for 24 hours to destroy soil microfauna such as *Collembola* sp which could otherwise graze on the mycelial systems.

2.8.3 *Inoculation of soil trays*

Pre-colonised wood inocula were removed from the 2l wide-necked conical flasks, each flask containing 30 wood blocks. Inocula were scraped free of surface mycelium and agar and were firmly pushed into the soil. Depending on experimental design, wood inocula were either placed centrally or 2cm from the edge of the tray set on the diagonal. Some inocula blocks were sacrificed to estimate the relative density and moisture content.

2.8.4 *Incubation of soil trays*

Inoculated trays were wrapped in polythene bags to minimise moisture loss. Trays were weighed between 7-12 day intervals throughout the experiment. Any water, which had evaporated, was replaced by spraying gently with distilled water around the edges of the soil systems. Soil trays were incubated in a constant temperature room at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the dark.

2.8.5 *Harvesting of mycelial systems*

At the end of the experiment, inocula and wood baits were separated from the mycelium and surface soil was removed. Fresh weights and volume were calculated to determine relative density and moisture content. Inocula and wood baits were oven dried for 72 hours at 80°C . Oven dry weights of wood components were recorded.

2.8.6 *Monitoring Phosphorus movement in mycelial systems*

All mycelial system to be analysed for phosphorus translocation received uniformly 64 x 0.05ul aliquots of 20 mM phosphorus solution this was to ensure that mycelial systems were gradient free. To the soil contained within the Petri dishes serving as radiotracer

compartments, aliquots of phosphorus solution containing ^{32}P orthophosphate (Amersham, carrier-free in dilute HCL) solution, depending on experimental design was added either around beech wood inocula colonised by cord-forming fungi or to the wood inocula pre-colonised by resource restricted fungi.

2.8.7 *Non Destructive monitoring of isotope with time*

Translocation of ^{32}P was monitored non-destructively with time using a PB 4 Anthracene scintillation probe (Nuclear Enterprises, Huntingdon, UK) attached to a scaler ratemeter (nuclear Enterprises PSR 8) recording counts per second over 30 seconds. The scintillation probe was positioned onto a Perspex moulding (Fig.2.1) to shield out background radiation around the wood bait. Radioactivity was determined in all wood resource types. Counts were recorded once a week for baits. To calculate background radiation levels in mycelial systems, counts in soil were also recorded once a week. Counts were corrected for decay using the formula below:

$$A_0 = A_t / e^{-0.693 (t/t^{1/2})}$$

Where	A_0	=	Initial activity
	A_t	=	Count
	t	=	day
	$t^{1/2}$	=	Half life of ^{32}P (14.3d)

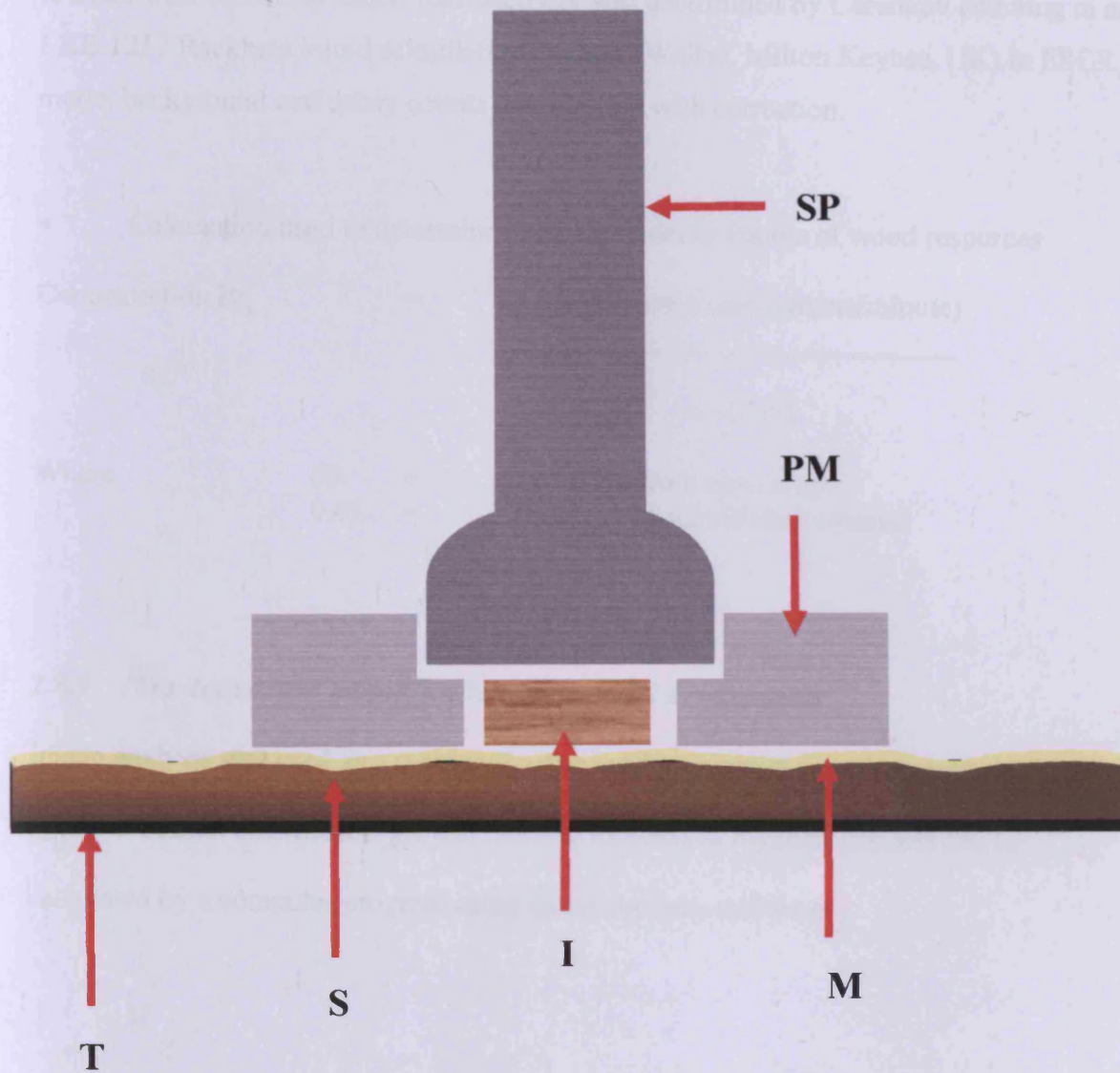


Figure 2.1 Diagram illustrating a section of Perspex tray (T) filled with compressed soil (S) with mycelial systems (M) foraging predominately on the upper layers of the soil. ^{32}P was monitored via a scintillation probe (SP) positioned and supported using Perspex moulding (PM) placed over a point of interest e.g. the inoculum (I) or wood resource.

2.8.8 *Destructive ³²P determination of mycelial systems*

At the end of the experiment, all wood components labelled with ³²P were ground by passing them through a Fritsch ‘pulverisette’ (Christison, Gateshead, UK). Wood samples were then digested in 4cm³ of 18m H₂SO₄ in a micro-Kjeldahl apparatus, diluted to 20ml with deionized water. Radioactivity was determined by Cerenkov counting in an LKB 1217 Rackbeta liquid scintillation counter (Wallac, Milton Keynes, UK) in ESCR mode, background and decay counts are reported with correction.

- Calculation used to determine destructive decay counts of wood resources

$$\text{Concentration Bq} = \frac{\text{Decay corrected cpm (counts/minute)}}{60 \times 0.45}$$

Where: 60 = conversion from cpm to cps
0.45 = Efficiency of scintillation counter

2.8.9 *Non destructive quantification of mycelial development*

Image analysis was used as a rapid technique used to quantify non-destructively mycelial systems. Fractal dimensions, growth rate and biomass of mycelia systems can be calculated by a computer program using image analysis software.

2.9 PHOTOGRAPHY AND IMAGE ANALYSIS

2.9.1 *Image capture of mycelial systems*

Inoculated soil trays were photographed from a fixed height of 90cm using a hitachi KP-M1 monochrome CCD video camera, fitted with a canon TV macro-zoom lens, linked to an Optipex GXMT 5100 microcomputer (Dell Systems Ltd, Wicklow, Republic of Ireland) with a Synapse framestore (synapse, Synoptics Ltd, Cambridge, UK.), Wells et

al (1998). The images of mycelial systems were captured and displayed on a second monitor to enable the use of SEMPER for windows version 6.1 image processing software (synoptics Ltd). Analogue monochrome signals from the video camera were converted to digital signals which are stored as a picture consisting of 512-512 black and white pixel arrays. Pixels can have a grey scale value between 0 (black) and 255 (white).

The captured mycelial images were analysed using SEMPER for Windows version 6 image processing software. Using appropriate commands in SEMPER, images were pre-processed to yield binary images, free of extraneous material. Firstly the High pass filter application was applied to the image to remove large range variation in intensity of the image. This was often due to unevenness in the level of soil, which can cause variation in light scattering throughout the image. This operation was achieved by subtracting the local mean grey value from each pixel over the surrounding 120-pixel square area.

The contrast between the mycelium and the surrounding soil was enhanced using the sharpening feature of SEMPER which automatically follows after high pass filtering. The sharpening feature doubles each pixel value and subtracts the local grey level measured over the surrounding square of 120×120 pixels. Histogram equalisation follows whereby pixels values were linearly re-scaled to the range of 0 (black) - 255 (white).

Manual adjustment of the threshold value was employed to produce a binary image (black and white). The threshold value was the same throughout the image analysis of mycelial systems. Removal of extraneous detail (by using a mouse) of the mycelial system such as baits and wood inocula was achieved by windowing out chosen areas.

Similarly the edge of the soil plates were also excluded from the analysis. All windowed out area's were set to pixel value 0 (black).

Particle analysis of the digital mycelial images using SEMPER enables the definition of particles comprising of 8 or more connected white pixels surrounded by black pixels.

Each particle within an image is allocated an identification code. Particles of 20 pixels or fewer were eliminated during the particle analysis phase, these particles represent reflective soil or grains.

2.9.2 *Image Analysis of mycelial systems*

Images were divided into sectors of interest to study hyphal development and polarity in different areas of the system. Firstly the image analysis software was calibrated, this was achieved by drawing a line of known length (cm) on the mycelial systems (e/g wood block length) and entering the information into the image analysis software programme. To calculate rate of radial increase over time in mycelial systems, lines were drawn using a mouse from the edge of the inocula to the end of the mycelial growing front . The information generated was automatically transferred to data files.

Fractal dimensions were determined as quantitative measures of space filling and progression or decline of mycelial growth of basidiomycetes (Boddy, 1999; Donnelly, Wilkins & Boddy,1995). Fractal structures do not possess the simple whole number dimensions of regular shapes such as circles and lines. Fractal structures have natural and more complex shapes. Two-dimensional structures have fractal dimension between 1 and

2, 1 being a line and 2 being a filled plane. Two fractal dimensions can be calculated for the mycelial images.

- 1 Border Fractal Dimension D_{BS} - sum of all perimeters of the captured image.
- 2 Mass Fractal Dimension D_{BM} - measurement of space filled of the captured image.

Fractal dimensions of mycelial systems were determined using the Box Counting method described by Donnelly *et al* (1995). The captured image was overlaid with a number of grids containing boxes ranging in side length between 3-61 pixels. Three types of boxes were counted overlaying the captured mycelial image: Interior boxes contain any white pixels (indicating mycelia), border boxes contain at least one white pixel (indicating spatial distribution of mycelia) and finally boxes containing only black pixels indicate that mycelia are absent.

Fractal dimensions was determined for the following relationship by plotting:

$$N(s) \approx cs^{-D_B}$$

where $-D_B$ = Box counting Fractal Dimension

$N(s)$ = Total number of boxes of side length (s) pixels intersected by N

An estimate of the Border Fractal of mycelial systems was obtained by plotting $\log N_{\text{border}}(s)$ against $\log s$. Regression analysis of the linear portion of the plot yields a gradient of $-D_{BS}$.

An estimate of mass fractal dimension can be obtained by regression analysis on the linear portion of the plot of:

$\{ N(s) - \frac{1}{2} N_{\text{border}}(s) \}$ against $\log s$ which yields a gradient of $-D_{\text{BM}}$

2.10 *Isolation of basidiomycetes at harvest*

To determine the degree of replacement, if any, in wood blocks at the end of the experiment, wood chips were removed and plated onto kanomycin agar. Each wood block was aseptically sliced in half to produce 2cm^3 blocks (Fig.2.2). Four chips were aseptically removed from each wood block and transferred on to a 9cm diameter Petri dish containing 2% MA. Petri plates were then incubated for 3 weeks at 20°C to allow mycelia to develop.

CHAPTER 3

Addition of colonised resources to established mycelial systems of

Phanerochaete velutina: Effect on mycelial development

3.1 INTRODUCTION

From studies in model soil systems in the laboratory, saprotrophic cord-forming basidiomycetes (e.g. *Phanerochaete velutina*) exhibit remarkable patterns of reallocation of biomass when foraging mycelia encounter new resources (Boddy, 1999). The new resources have usually been un-colonised wood blocks. However, in natural ecosystems, suitable (i.e. dead) resources encountered by foraging mycelia of saprotrophs are almost always already colonised, since twigs, branches and trunks are usually well decayed prior to fall (Boddy, 2001).

In forest and woodlands, saprotrophic cord-forming fungi, including *P. velutina*, often form systems that are extensive (distributed over several to many metres or even hectares) and long lived (Boddy, 1993; Boddy, 1999; Smith, Bruhn & Anderson, 1992; Thompson, 1984). Therefore, as well as locating new resources by foraging, resources can arrive by falling directly onto an established system. Effects on reallocation of biomass vary depending on whether it is a foraging front or mature part of the system that encounters the new resource (Wells, Harris & Boddy, 1998a). Thus, for example, there was much less regression of fine mycelium when a resource was encountered at the front compared with encounter by more mature regions.

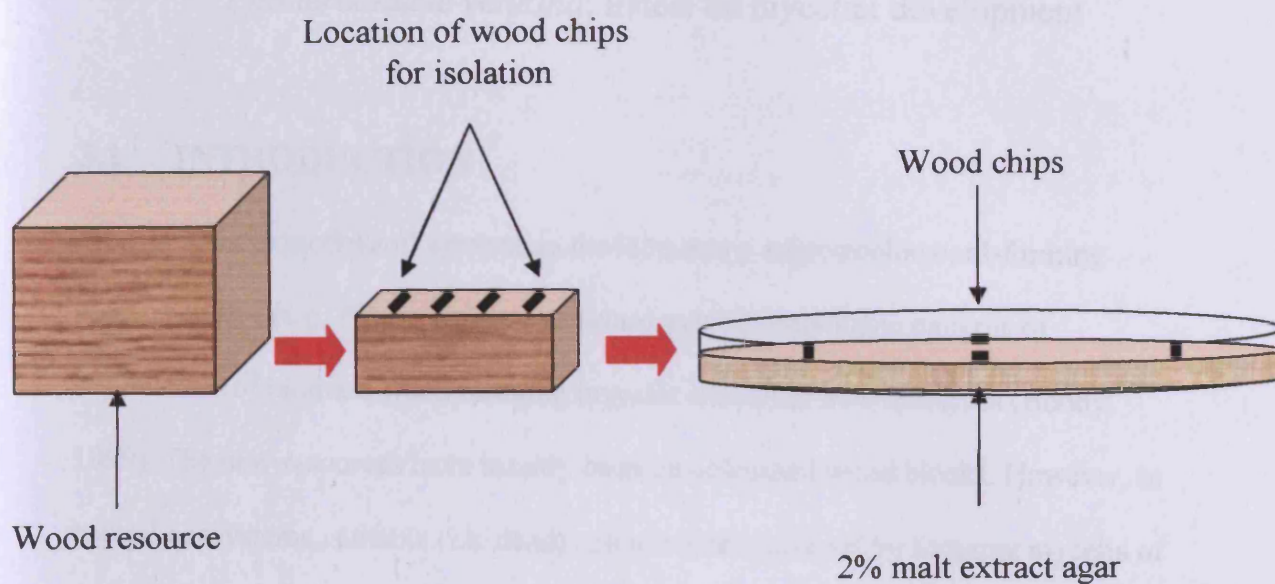


Figure 2.2 Diagram showing the stages in aseptic isolation of wood chips from wood inocula and baits.

CHAPTER 3

Addition of colonised resources to established mycelial systems of

Phanerochaete velutina: Effect on mycelial development

3.1 INTRODUCTION

From studies in model soil systems in the laboratory, saprotrophic cord-forming basidiomycetes (e.g. *Phanerochaete velutina*) exhibit remarkable patterns of reallocation of biomass when foraging mycelia encounter new resources (Boddy, 1999). The new resources have usually been un-colonised wood blocks. However, in natural ecosystems, suitable (i.e. dead) resources encountered by foraging mycelia of saprotrophs are almost always already colonised, since twigs, branches and trunks are usually well decayed prior to fall (Boddy, 2001).

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This study aims to determine whether the presence of another fungus occupying an encountered resource affects mycelial characteristics in foraging systems of *P. velutina* to which new resources have been added in mature regions, as opposed to at a foraging front.

The hypothesis is that mycelial response to addition of wood colonised by a relatively poor combatant e.g. *Vuilleminia comedens*, is similar to that when uncolonised wood is added but response to wood precolonised with a more aggressive fungus will be different.

3.1 MATERIALS AND METHODS

3.2.1 Fungal isolates

Phanerochaete velutina, *Stereum hirsutum*, *Coriolus versicolor*, *Phlebia radiata* and *Vuilleminia comedens* were originally isolated from decaying beech wood from the Forest of Dean, UK, (National Grid Reference SO 611145). Laboratory stocks were routinely maintained and routinely subcultured as described in section 2.1- 2.2.

3.2.2 Preparation of Inocula Wood Blocks

Wood blocks (20×20×10 mm³) were cut from freshly felled beech and were stored in a freezer at -18°C until required. The appropriate number of wood blocks needed were defrosted, soaked overnight in deionised water and autoclaved twice for 30 minutes at 121°C at 24 hour intervals. Batches of 30 wood blocks were added to 2 l conical flasks containing two week old cultures of *P. velutina* grown on 500ml 2% w/v malt extract

agar. Two weeks later autoclaved wood blocks were added to two week old cultures of *Stereum hirsutum*, *Coriolus versicolor*, *Phlebia radiata* and *Vuilleminia comedens*. The flasks were then incubated at 20°C for 8 weeks to ensure colonisation of the wood blocks.

3.2.3 Preparation and Maintenance of Soil Trays

Non-sterile soil was used. Soil was collected from a mixed deciduous woodland (Tintern, UK; National Grid Reference SO 528018). Coarse leaf and wood litter were removed and the soil was sieved through a 10mm mesh before air drying in the laboratory. Air dry soil was sieved through 5mm and 2mm mesh to remove organic matter and frozen for 24 h at -18°C to destroy soil microfauna such as *Collembola* sp. which could otherwise interfere with the experimental analysis by grazing the mycelial systems. Soil matric potential of the bulk mixture was adjusted to -0.012MPa (Fawcett & Collis-George, 1967) by addition of deionised water. Prepared Soil (600g) was compressed evenly into 24 × 24 cm² plastic bioassay trays (Nunc: Gibco, Paisley, UK) so that mycelial growth would be predominately on the surface of the soil.

3.2.4 Effect of the addition of a single pre-colonised wood resource on pattern formation in mycelial cord systems

Beech wood blocks precolonised by *P. velutina* were placed centrally on compressed soil and trays were incubated in darkness at 20°C for 23 d to ensure sufficient growth of mycelia to allow wood resource types to be positioned behind the mycelial margin. Single pre-colonised wood resources either colonised for 8 weeks with *Stereum*

hirsutum, *Coriolus versicolor*, *Phlebia radiata* or *Vuilleminia comedens* were positioned on a diagonal from *P. velutina* inocula, 2cm away from the edge of the tray. A fresh previously un-colonised wood resource was used as a control (positive controls), and a second control had no additional resources (negative controls). Five replicates were made of each treatment. The trays were kept in polythene bags to minimise moisture loss and were incubated in the dark at 20°C. Morphology of *P. velutina* was quantified by determining fractal dimension and hyphal cover, for 155 d at 7-12 d intervals, as described in section 2.9.1 – 2.9.2.

3.2.5 Decay rate and colonisation of beech wood resources

Before the start of the experiment the initial relative density (RD g cm⁻³) of all inocula was obtained (Table 3.1). At 155 days mycelial systems were harvested and the final RD was determined in order to estimate wood decay rates (g mg³d⁻¹) for all inocula and wood resources. Wood chips from a sub-sample of pre-colonised wood resources and previously un-colonised resources were plated aseptically onto 2% Malt Extract Agar, as described in section 2.10, to determine outcome of mycelial interactions in wood.

3.2.6 Statistical analyses

One-way ANOVA and where appropriate *Posteriori* tests were used to compare treatment means, following Levene's test for equality of variances. Kruskal-Wallis tests were employed to compare treatment medians with unequal variances.

Paired t tests were performed when comparing data obtained from the baited sector of the mycelial systems with non-baited sectors. Wilcoxon's signed rank test was employed to compare treatment medians with unequal variances. Statistical analyses were performed using MINTAB for windows version 13.

Table 3.1 Relative densities of *Phanerochaete velutina*, pre-colonised and un-colonised wood resources at the start of the experiment

Wood resource un-colonised or colonised by:	Relative Density at start of experiment (g cm ⁻³)
<i>P. velutina</i>	0.578
<i>C. versicolor</i>	0.7235
<i>V. comedens</i>	0.8631
<i>P. radiata</i>	0.8371
<i>S. hirsutum</i>	0.7697
un-colonised	0.6068

3.3 RESULTS

3.3.1 *Qualitative outgrowth patterns*

Mycelial egressed from beech wood resources by 3 d, the start of cord formation was evident by 6 d (Fig. 3.1). The formation of well defined cords was observed at 10 d (Fig.3.1), cords continued to extend until they had reached the edge of the soil tray by 23 d, at which time wood resources were placed behind the foraging mycelial margin.

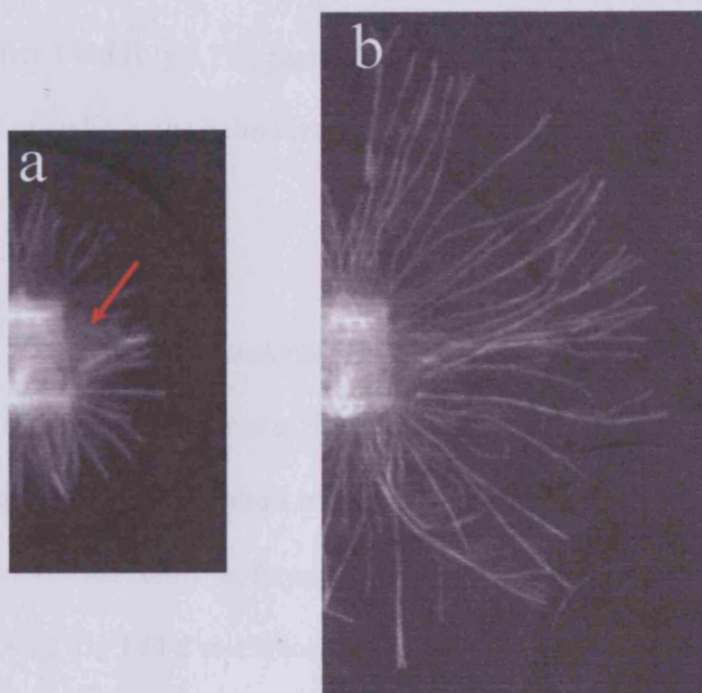


Figure 3.1 Mycelial systems of *Phanerochaete velutina* exhibiting: initial dense mycelial formation (indicated by arrow) egressing from the wood resource following aggregation into cords at 6 d (a) and established cord systems at 10 d (b).

3.3.1.1 *P. velutina* encountering the pre-coloniser *Coriolus versicolor*

After 50 d (Fig 3.2c) slight regression of mycelia occurred around the mycelial margin. After 64 d (Fig 3.2d-g) thickening of mycelial cords was evident. Substantial regression was evident after 144d (Fig 3.2h), particularly in the unbaited sector at the same time re-exploration of mycelia in the baited region of the system occurred.

3.3.1.2 *P. velutina* encountering the pre-coloniser *Stereum hirsutum*

Regression of mycelia was evident at 50 d (Fig 3.3c), however by 64 d, thick mycelial cords connected to the pre-colonised resource was observed. By 78 d cords had grown out of the pre-colonised resource. Mycelial regression in un-baited areas continued until 113 d (Fig 3.3 d-g). By 144 d re-exploration by mycelia was evident away from the pre-colonised wood resource (Fig 3.3h-I).

3.3.1.3 *P. velutina* encountering the pre-coloniser *Vuilleminia comedens*

Mycelial cords thickened following colonisation of the wood resource pre-colonised by *V. comedens*. Slight mycelial regression occurred at 64 d (Fig 3.4d) in un-baited areas of the system, and patchy mycelial growth was observed. Regression in un-baited areas continued until systems were harvested and dense mycelial growth from the pre-colonised wood resource was evident (Fig 3.4e-i). By 144 d cords had grown out of the pre-colonised wood resource (Fig 3.4h).

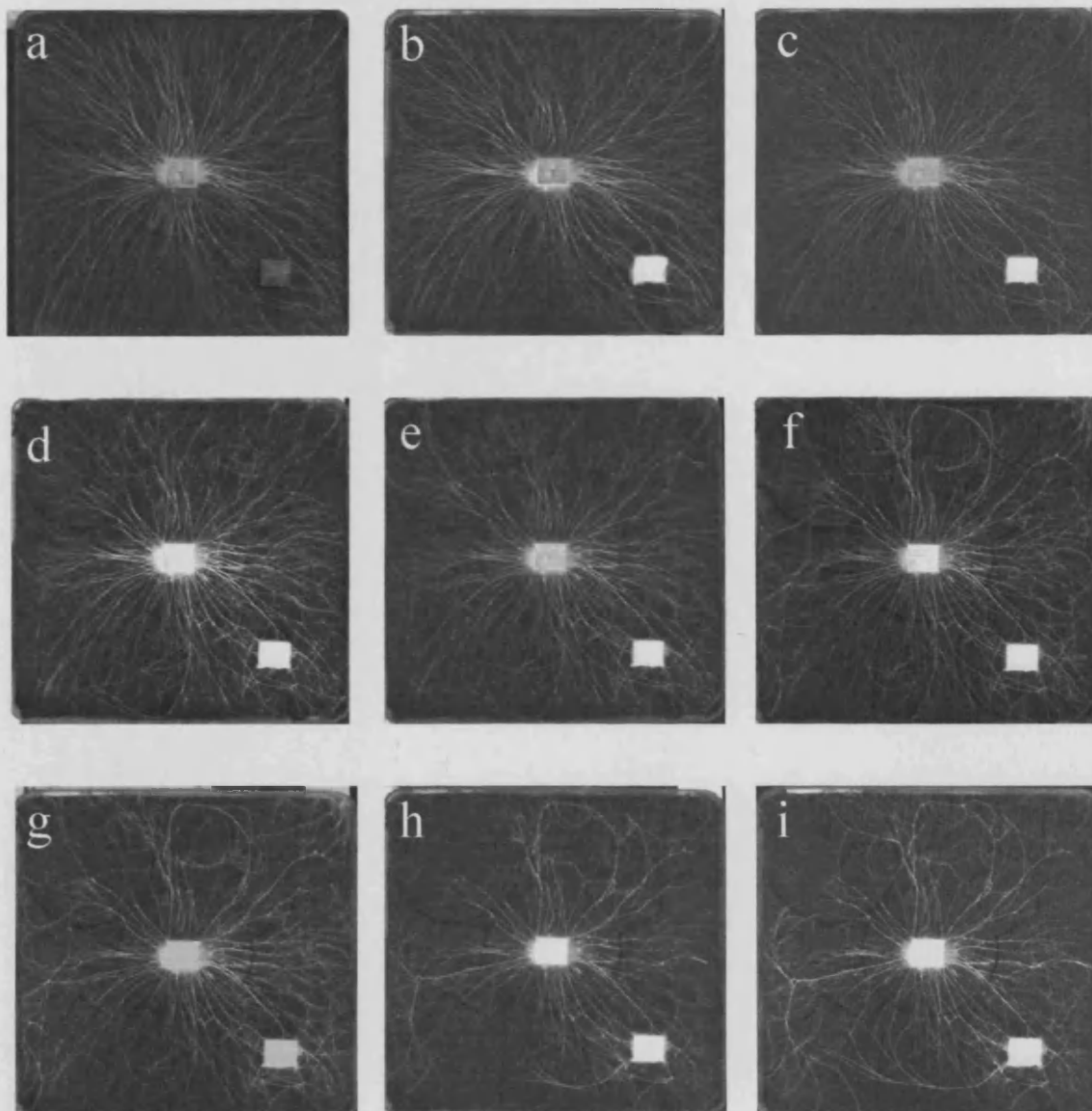


Figure 3.2 Mycelial systems of *Phanerochaete velutina* encountering a pre-colonised wood resource containing *Coriolus versicolor* at 23(a), 36(b), 50(c), 64(d) 78(e), 101(f), 113(g), 144(h) and 155(i) days.

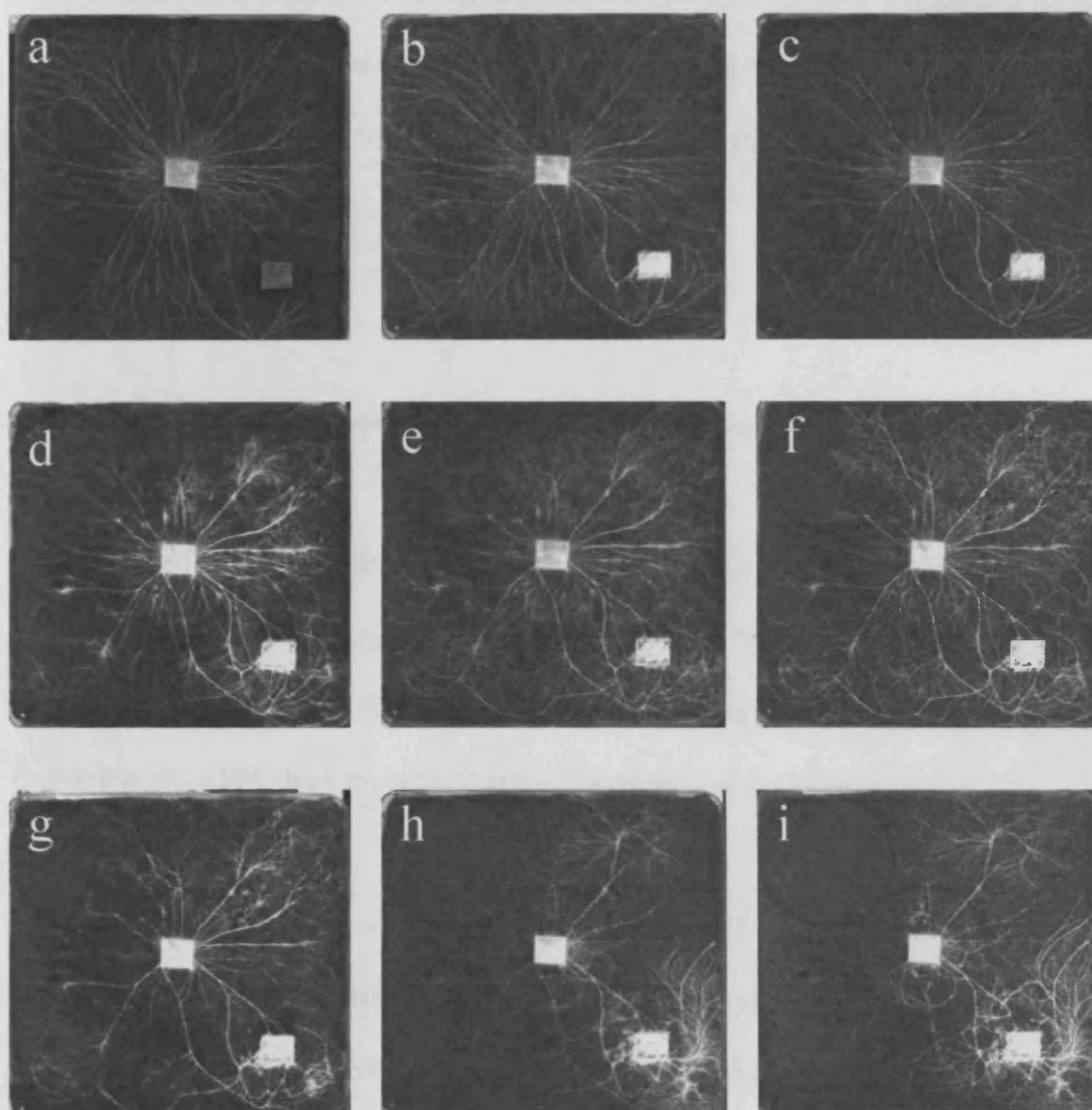


Figure 3.4 Mycelial systems of *Phanerochaete velutina* encountering a pre-colonised wood resource containing *Vuilleminia comedens* at 23(a), 36(b), 50(c), 64(d) 78(e), 101(f), 113(g), 144(h) and 155(i) days.

3.3.1.4 *P. velutina* encountering the pre-coloniser *Phlebia radiata*

Regression of fine mycelia leaving thick cords was evident by 50 d (Fig 3.5c).

Thickening of mycelial cords contacting the pre-colonised resource was evident between 64- 155 d (Fig 3.5d-h). There was no obvious regression in the area of the system not directly in contact with *P. radiata*. Cords grew out from the pre-colonised resource by 144 d (Fig 3.5g).

3.3.1.5 *P. velutina* encountering a previously un-colonised wood resource (positive controls)

Thickening of cords extending between wood resources occurred by 36 d (Fig 3.6b), followed by some mycelial regression of fine mycelia by 50 d, leaving thickened radial cords (Fig.3.6c). Extensive mycelial regression occurred in the un-baited area (Fig 3.6 d-i), leaving just a few thick mycelial cords connecting to wood resources.

3.3.1.6 Negative controls of *P. velutina* mycelial systems

Well-established mycelial systems persisted for over 100 d but by 144 d far fewer cords remained (Fig 3.7h).

3.3.2 Hyphal coverage and radial extension

Initially, mycelium extended from inocula at $0.57 \pm 0.05 \text{ cm d}^{-1}$. Measurements after 23 d were precluded by the mycelium reaching the edges of the soil trays. Rates of total

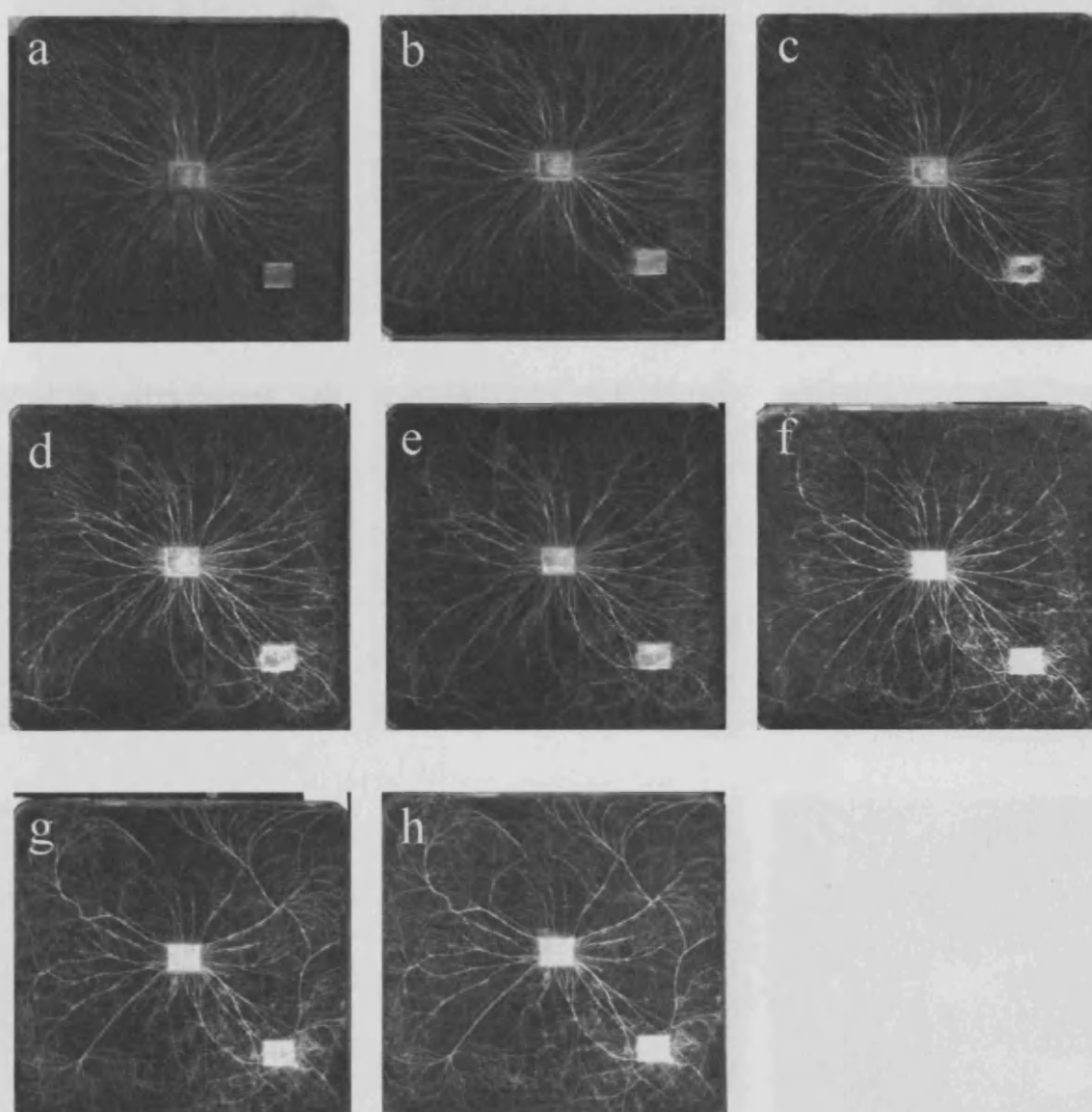


Figure 3.5 Mycelial systems of *Phanerochaete velutina* encountering a pre-colonised wood resource containing *Phlebia radiata* at 23(a), 36(b), 50(c), 64(d) 78(e), 113(f), 144(g) and 155(h) days.

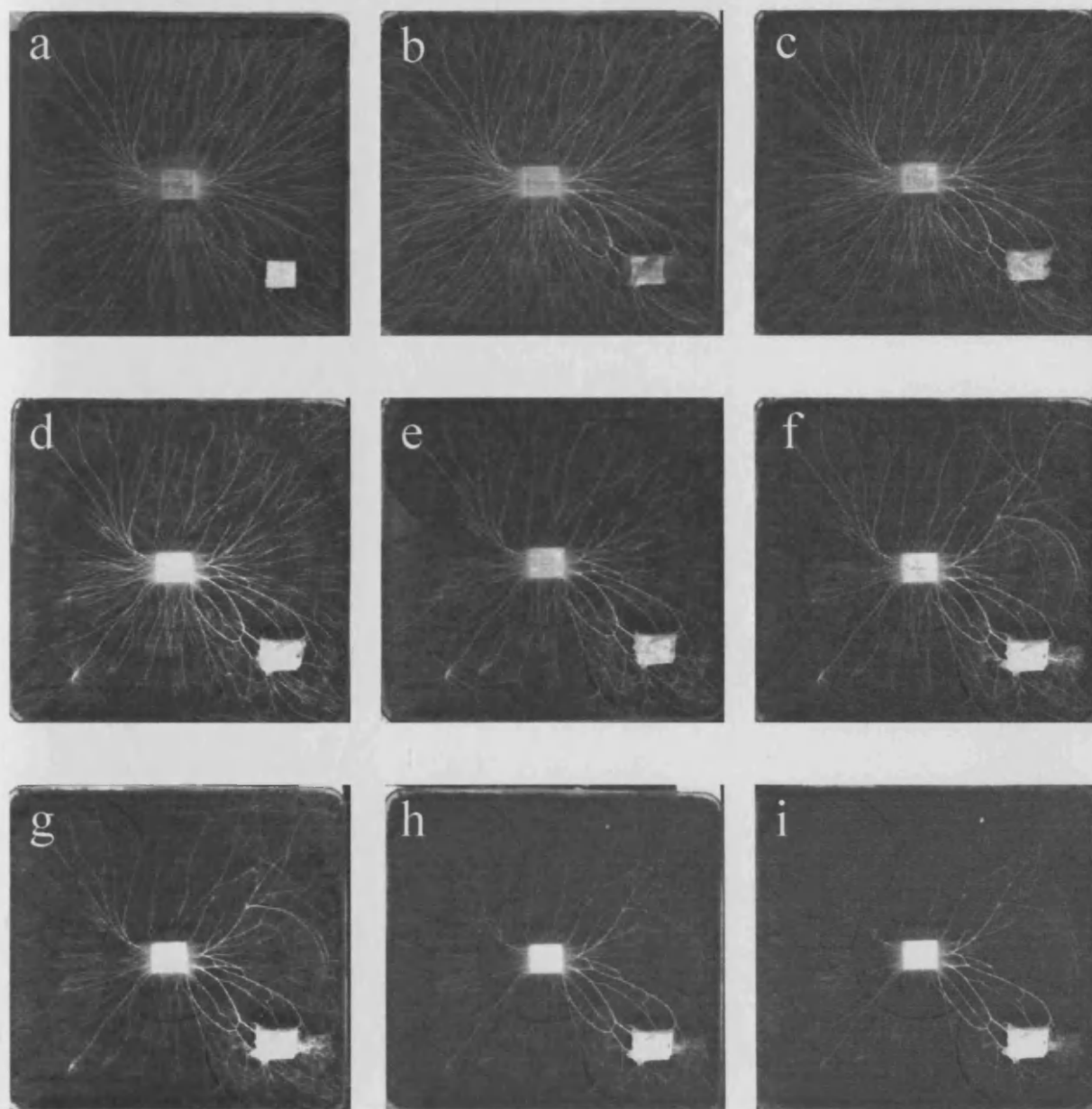


Figure 3.6 Positive controls of mycelial systems of *Phanerochaete velutina* encountering a previously un-colonised fresh wood resource at 23(a), 36(b), 50(c), 64(d) 78(e), 101(f), 113(g), 144(h) and 155(i) days.

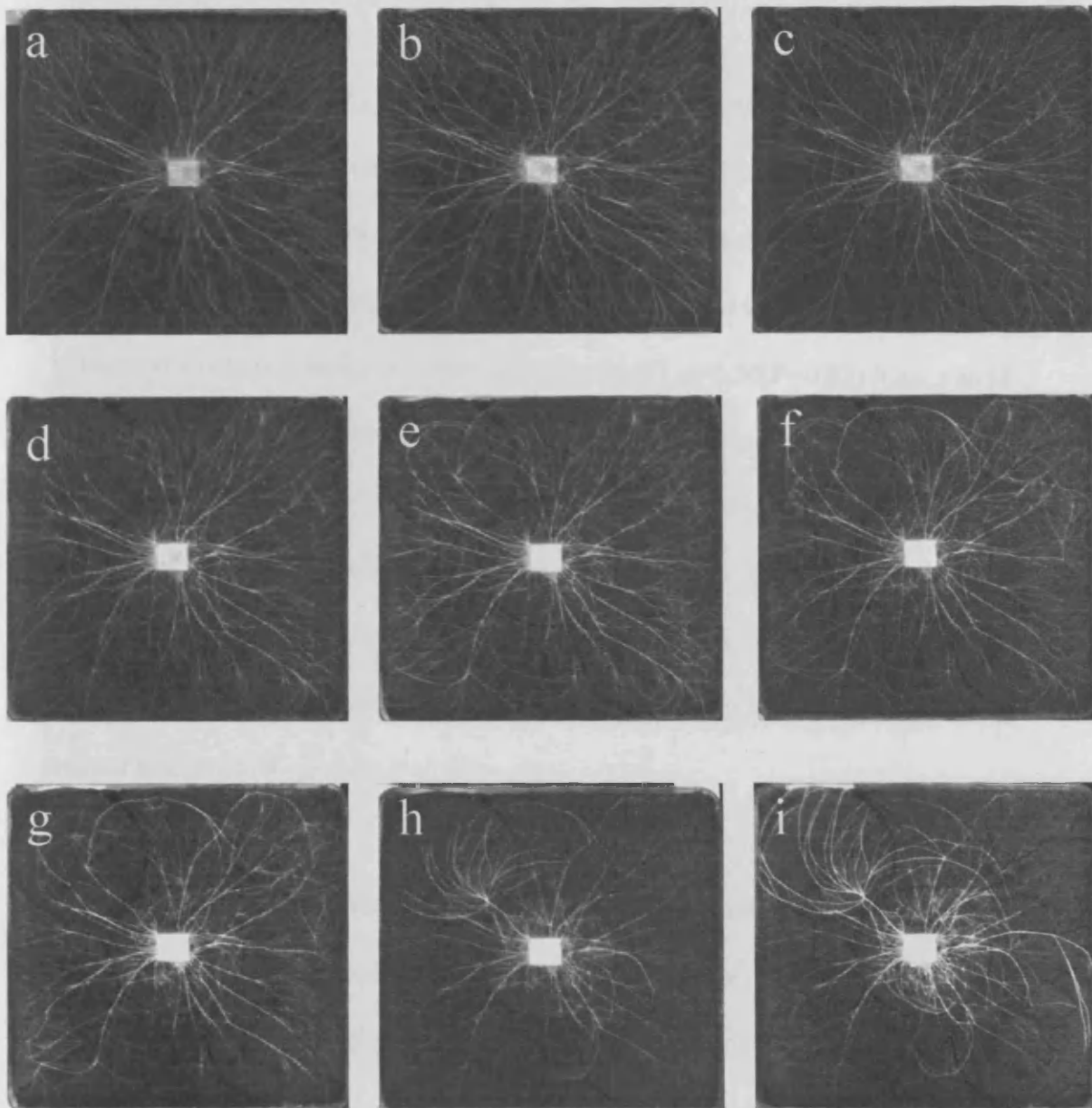


Figure 3.7 Negative control mycelial systems of *Phanerochaete velutina* at 23(a), 36(b), 50(c), 64(d) 78(e), 101(f), 113(g), 144(h) and 155(i) days.

hyphal cover appeared to be exponential until 23-36 d after which rates of hyphal cover gradually declined (Fig 3.8).

There was a significant ($F_{5,24}=2.88, P<0.05$) effect of addition of pre-colonised / previously un-colonised wood resources to mycelial systems of *P. velutina* at 36 d; hyphal coverage in negative control systems was significantly higher than systems interacting with wood resources pre-colonised by *Coriolus versicolor*. Hyphal coverage in negative control systems was also significantly ($F_{5,24}=3.50, P<0.05$) higher at 50 d compared to systems of *Phanerochaete velutina* encountering wood resource pre-colonised by *Vuilleminia comedens*, *Phlebia radiata* and *Stereum hirsutum*. Hyphal coverage in all systems declined between 36-50 d (36-64 d in the case of negative controls). At 101d hyphal coverage in systems colonising a previously un-colonised wood resource was significantly lower than mycelial systems encountering *Phlebia radiata* resources ($F_{5,24}=2.64, P<0.05$).

A comparison of mean surface hyphal coverage between baited and un-baited sectors was performed by electronically sectioning areas of interest using SEMPER software, as described in section 2.9.2. Significant differences between the means of baited and un-baited regions of the mycelial systems were detected ($P<0.05$), the baited sector usually having a greater hyphal coverage (Fig. 3.9). Surface hyphal coverage in systems encountering previously un-colonised wood resources peaked at day 78 and then declined (Fig.3.9e).

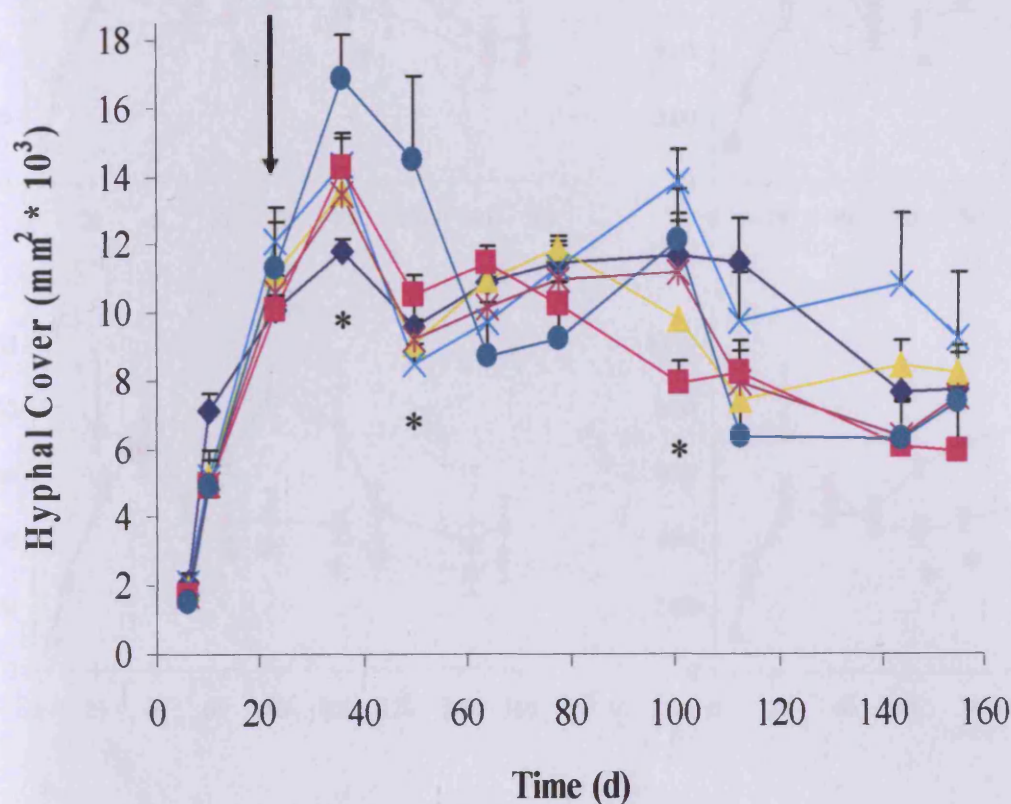


Figure 3.8 Change in mean surface hyphal coverage with time in mycelial systems of *Phanerochaete velutina* growing on unsterile soil. Systems were supplied with pre-colonised wood resources containing either *Coriolus versicolor* (◆), *Vuilleminia comedens* (▲), *Phlebia radiata* (×) and *Stereum hirsutum* (*). Positive control systems were supplied with a fresh previously un-colonised beech wood resource (■) and negative control systems were without a wood resource(●). Arrows indicate time of addition of pre-colonised wood resources. * denotes significant difference ($P < 0.05$) between means, following one-way ANOVA and priori tests.

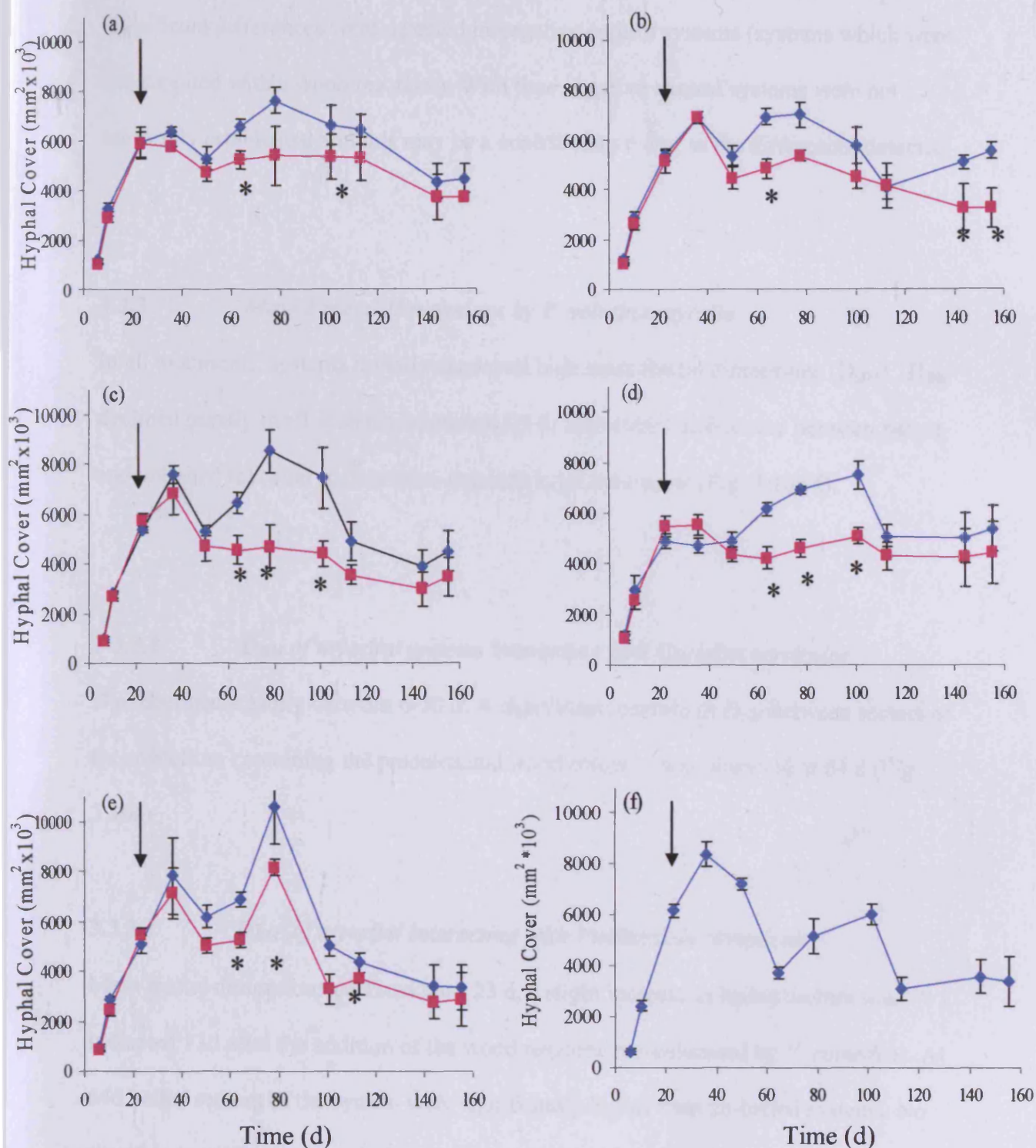


Figure 3.9 Mean surface hyphal coverage with time in baited (◆) and un-baited (■) sectors of mycelial systems of *Phanerochaete velutina* growing on un-sterile soil. Systems were supplied with pre-colonised wood resources containing either *Coriolus versicolor* (a), *Vuilleminia comedens* (b) *Stereum hirsutum* (c) *Phlebia radiata* (d). Positive control systems were supplied with a fresh previously un-colonised beech wood resource (e) and negative control systems were without a wood resource (f). Arrows indicate time of addition of pre-colonised wood resources. * denotes significant difference (P < 0.05) between means, following paired t tests.

Significant differences were detected in negative control systems (systems which were not supplied with a wood resource). With time negative control systems were not uniformly established and this may be a contributing factor to the differences detected.

3.3.3 *Mass Fractal Dimensions by *P. velutina* mycelia*

In all treatments, systems initially displayed high mass fractal dimensions (D_{BM}). D_{BM} declined greatly in all systems between 6-23 d. Significant differences between baited and unbaited mycelial sectors were detected in all treatments (Fig. 3.10a-f).

3.3.3.1 *D_{BM} of mycelial systems interacting with *Coriolus versicolor**

D_{BM} declined steadily between 6-50 d. A significant increase in D_{BM} between sectors of the mycelium containing the precolonised wood resource was observed at 64 d (Fig 3.10a).

3.3.3.2 *D_{BM} of mycelial interacting with *Vuilleminia comedens**

Mass fractal dimensions declined until 23 d, a slight increase in baited sectors was indicated 13d after the addition of the wood resource pre-colonised by *V. comedens*. At 64d baited sectors of the system were significantly higher than un-baited systems. No significance differences were detected between sectors until 155d, when baited sectors were much higher than un-baited sectors (Fig 3.10b).

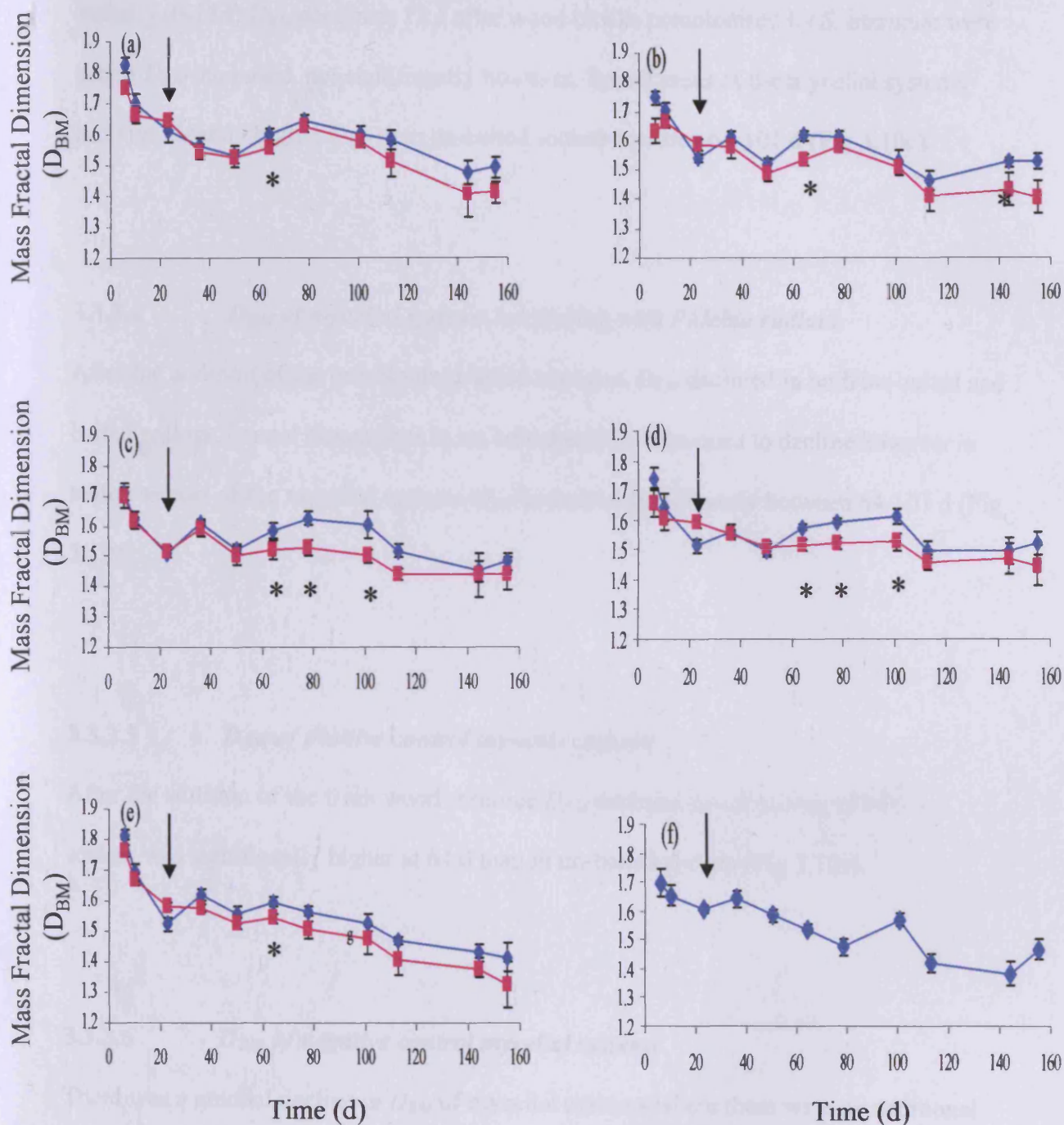


Figure 3.10 Change in time in mass fractal dimensions (D_{BM}) in baited (◆) and un-baited (■) sectors in mycelial systems of *Phanerochaete velutina* encountering pre-colonised resources containing *Coriolus versicolor* (a), *Vuilleminia comedens* (b), *Stereum hirsutum* (c) and *Phlebia radiata* (d). Positive control systems were supplied with a fresh previously un-colonised beech wood resource (e), and negative control systems were without a wood resource (f). Arrows indicate time of addition of pre-colonised wood resources. * denotes significant difference ($P < 0.05$) between means, following paired t tests.

3.3.3.3 D_{BM} of mycelial systems interacting with *Stereum hirsutum*

Initially (0-23d) D_{BM} declined; 13 d after wood blocks precolonised by *S. hirsutum* were added D_{BM} increased, not significantly however. Baited areas of the mycelial systems had significantly higher D_{BM} than un-baited sectors between 64-101 d (Fig 3.10c).

3.3.3.4 D_{BM} of mycelial systems interacting with *Phlebia radiata*

After the addition of the precolonised wood resource, D_{BM} declined in both un-baited and baited sectors. Fractal dimensions in un-baited sectors continued to decline however in baited sectors of the mycelial systems D_{BM} increased significantly between 64-101 d (Fig 3.10d).

3.3.3.5 D_{BM} of positive control mycelial systems

After the addition of the fresh wood resource D_{BM} declined steadily, D_{BM} of baited sectors was significantly higher at 64 d than in un-baited sectors (Fig 3.10e).

3.3.3.6 D_{BM} of negative control mycelial systems

There was a gradual decline in D_{BM} of mycelial systems where there were no additional wood resources added to the systems Fig 3.10.

3.3.4 Wood decay rate and percentage weight loss at harvest of *Phanerochaete velutina* inocula and pre-colonised resources

No significant difference ($P>0.05$) was detected in the relative wood decay rate of *Phanerochaete velutina* inocula encountering different pre-colonised wood resources (Table 3.2). However, there was significance in the relative wood decay rate of pre-colonised inocula. Treatments where *P. velutina* encountered a previously un-colonised resource, the relative wood decay rate of the inoculum was significantly lower ($P<0.05$) than against all other unit restricted fungi. No significant differences were detected in between treatments and resource types in relation to percentage weight loss of *P. velutina* inocula, wood resources precolonised and total weight loss in systems (Appendix 1).

Pre-colonised baits by wood decay species	Relative wood decay rate of pre-colonised baits (mg g ⁻¹ d ⁻¹)	Relative wood decay rate of inocula (mg g ⁻¹ d ⁻¹)
Negative controls	absent	absent
Positive controls	1.78 ± 0.19 ^b	4.193 ± 0.04 ^a
<i>Coriolus versicolor</i>	4.10 ± 0.16 ^a	3.990 ± 0.19 ^a
<i>Stereum hirsutum</i>	3.21 ± 0.12 ^a	3.673 ± 0.23 ^a
<i>Phlebia radiata</i>	3.99 ± 0.27 ^a	3.786 ± 0.33 ^a
<i>Vuilleminia comedens</i>	3.39 ± 0.20 ^a	3.852 ± 0.11 ^a

Table 3.2 Data represented are a summary for 5-10 replicate mycelial systems. Decay data presented with the standard error of the mean ($n = 5-10$). Figures in the same column followed by the same letter are not significantly different ($P>0.05$) following one-way ANOVA and *Priori* tests.

3.3.5 Outcome of mycelial interactions in pre-colonised wood following a encounter by *P. velutina*

At the end of the experiment (155 d) resources that were pre-colonised by *Vuilleminia comedens* were fully replaced by *P. velutina* (Table 3.3). *P. velutina* partially replaced two resources already colonised by *Phlebia radiata*, while in the remaining three there was no evidence of *P. radiata* mycelia present. Four resources previously colonised by *Coriolus versicolor* were fully replaced by the cord former; in the remaining resource it was only partially replaced. Of the four pre-colonised resources encountered by *P. velutina*, *Stereum hirsutum* was the only fungus able to withstand “attack” by *P. velutina*. In 3 resources there was no evidence of colonisation by *P. velutina*. In the remaining two resources however, it was only partially colonised by *P. velutina*.

Pre-coloniser	Percentage colonisation by <i>P. velutina</i> of pre-colonised wood resources (%)				
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
<i>Coriolus versicolor</i>	100	100	100	100	25
<i>Stereum hirsutum</i>	75	50	0	0	0
<i>Vuilleminia comedens</i>	100	100	100	100	100
<i>Phlebia radiata</i>	100	100	100	75	75

Table 3.3 Outcome of interactions between *P. velutina* and pre-colonised wood resources

The extent of replacement of resource restricted fungi varied between the species investigated in decreasing order as follows: *Vuilleminia comedens*, *Phlebia radiata*, *Coriolus versicolor* and *Stereum hirsutum*.

3.3 DISCUSSION

In this study I quantified and visualised the development of mycelial systems of *Phanerochaete velutina* interacting with unit restricted fungi; *Coriolus versicolor*, *Phlebia radiata*, *Stereum hirsutum* and *Vuilleminia comedens*, already present in beech wood. This appears to be the first visualisation and quantification of mycelial growth using fractal geometry to study interactions with unit restricted fungi. Fractal geometry has been employed to detect changes in the morphological development of *P. velutina* whilst being subjected to competition for carbon resources.

In the only study examining the effects of resources already colonised by other fungi, (these resources were placed ahead of the mycelial colony), there were some evidence of differences in response of mycelial systems of *P. velutina*. This depended on whether or not the resources were colonised and which fungus was present in the resource (Boddy & Abdalla, 1998).

The present study has shown for the first time that when a resource arriving (placed behind the growing margin of the mycelial colony) on an established system is already colonised, the presence of a fungus can have major effects on deployment of biomass by the established system. Effects are specific to species occupying the resource.

The results show that initial outgrowth from beech wood inocula was similar to that reported in previous studies conducted by Wells, Harris & Boddy (1998a).

Development did differ, however; initial colony formation appeared to be symmetric as opposed to asymmetric. However by analysing surface hyphal coverage with time, significant differences were detected in mature systems after 36-50 d, particularly systems lacking a precolonised resource.

On arrival of precolonised resources and previously uncolonised resources by mycelial systems of *P. velutina*, thickening of connective mycelial cords was evident. Polarisation has been commonly detected in previous studies encountering both precolonised and uncolonised wood resources where wood was added in front of the colony margin (Wells, Harris & Boddy 1998a). Polarisation was detected in this study between 64-155 d.

Hyphal coverage rates were reduced by the addition of wood resources to mycelial systems. A significant reduction in hyphal coverage occurred at 10 d in mycelial systems where wood resources previously uncolonised were introduced. This may be due to the lack of competition within the wood resources encountered by *P. velutina*, where redeployment of biomass by *P. velutina* was not required.

Redeployment of biomass is commonly seen when foraging fronts of mycelial systems in soil encounter a new uncolonised resource (Boddy, 1993; Boddy, 1999; Dowson, Rayner & Boddy, 1986; Dowson, Rayner & Boddy, 1988a); This was also seen (evidenced visually and by estimated of hyphal coverage) here when uncolonised resources were added to a developed system, i.e. distal to mycelial fronts. In previous

studies with *P. velutina*, however, redeployment of biomass was only obvious to the unaided eye when new resources were considerably larger than the inoculum from which the colony was developing (Dowson, Rayner & Boddy, 1986). In the present study, the new resource and the inoculum were the same size, implying that with this fungus the mycelial system responded more to resources added behind growing fronts than to those added in front. However, inocula were only half the volume (4cm^3 as opposed to 8cm^3) of those in the original study (Dowson, Rayner & Boddy, 1988a), which may be significant, as the inoculum size and the inoculum : new resource ratio affects mycelial characteristics and time before outgrowth from new resources (Bolton, 1993). A further difference is that systems were allowed to develop for much longer (155 d) than the earlier study (77 d) but even in the present study there was visual evidence that at 78 d, regression of mycelium not connecting with the inoculum and the new resource occurred. Increase in fractal dimension and hyphal coverage in the half of the system with the new resource compared to the half without, supports earlier findings (Wells, Harris & Boddy, 1998a), and further emphasises the redistribution of mycelial biomass when a new resource arrives on an established system.

In general mycelial systems were initially were highly mass fractal ($D_{\text{BM}}=1.8$). The addition of wood resources caused a significant effect on the mycelial pattern exhibited by *P. velutina* often resulting in polarised growth of mycelial systems. In all treatments, sectors of the mycelial system containing precolonised/uncolonised resources displayed a significant increase in fractal dimension when compared to that of sectors of the mycelial systems without a wood resource.

Differences in redeployment of mycelial biomass, depending on whether a new resource is unoccupied or occupied and on the precolonising species, appears to reflect the ease/difficulty with which *P. velutina* was able to colonise the new resource. As hypothesised, mycelial response to added wood occupied by poor combatants was similar to that of uncolonised wood. Thus, there was clear visual evidence of regression of non-connective mycelium in many systems containing new resources precolonised by *V. comedens*, these systems were relatively easily replaced, but no such redeployment of biomass was evident in systems containing a new resource precolonised by *C. versicolor*, *P. radiata* and *S. hirsutum*, which were more aggressive combatants. This ties in with previous observations that, when given a 'choice', *P. velutina* will preferentially invade wood blocks containing the least combative fungus (Boddy & Abdalla, 1998).

Differences in rate and extent of redeployment of biomass would be expected if different inoculum to new resource ratios were employed both because, as indicated above, this has already been shown to occur with uncolonised resources encountered by a foraging front (Dowson, Rayner & Boddy, 1986), and because relative size of resources can affect the outcomes of interactions (Holmer & Stenlid, 1993).

In conclusion, the presence of a fungus occupying a resource arriving on an established mycelial system in soil clearly has major effect on deployment of biomass. These effects are specific to species occupying the resource, and probably also to the species forming the established mycelium on the soil.

CHAPTER 4

Phosphorus translocation in mycelial systems of

Phanerochaete velutina: Effect of resource quality

4.1 INTRODUCTION

Studies on mycelial interactions between *P. velutina* and unit restricted fungi have shown major redeployment of biomass exhibited by *P. velutina* (Boddy & Abdalla, 1998; Chapter 3). This is dependent on species occupied within the resource that *P. velutina* is encountering. Qualitative studies have shown preferential colonisation by *P. velutina*, when simultaneously encountering two or three wood resources which were either uncolonised or precolonised by other wood decay fungi (Boddy & Abdalla, 1998) *P. velutina* preferentially colonised the resource offering least resistance; i.e. the resource containing a less combative fungus or no fungus at all.

Quantitative studies (chapter 3) have shown that significant changes in the fractal pattern exhibited by *P. velutina* was detected when a resource precolonised by other wood decay fungi or previously uncolonised arrived onto an already established mycelial system, often resulting in polarised growth towards the sector of the system containing the precolonised/ uncolonised wood resource. Hyphal coverage rates in mycelial systems were also significantly reduced by the addition of a single precolonised/uncolonised wood resource (Chapter 3).

A smaller earlier study also indicated that precolonisation of a resource can affect translocation of phosphorus to that resource, and this varied depending on species (*Xylaria hypoxylon* or *Stereum hirsutum*) (Wells, Hughes & Boddy, 1990).

In the aforementioned experiments the colonised resources were encountered by a growing mycelial front. Effects of such encounter may be different from the situation in which a new resource lands on a developed mycelial system. In a previous study mycelial systems foraging on a nutrient poor soil, mycelial 'patches' (comprising fine, highly branched separate hyphae extending radially from points of aggregated hyphae in cords) developed throughout systems when new uncolonised resources were added to mature regions (Wells, Donnelly & Boddy, 1997). The patches were sites of increased nutrient uptake, and were temporary structures, regressing more rapidly with age than other parts of the systems. Systems to which new resources had been added also displayed significant developmental polarity: total hyphal cover (patches plus cords) and hyphal patches were much greater in the part of the system containing the new resource. Phosphorous uptake and translocation were also dramatically affected by adding one or more new resources to mature systems: there was net translocation to relatively recently arrived (not necessarily most recent) resources at the expense of resources that had been incorporated into the system for longer (Wells, Boddy & Donnelly, 1998; Wells, Harris & Boddy, 1998b; Wells, Harris & Boddy, 1999).

This study aimed to answer the following questions for foraging mycelial systems to which one or two new resources have been added in mature regions, as opposed to at a foraging front: (1) does the presence of another fungus occupying an encountered

resource affect phosphorus uptake and allocation?; (2) are any effects specific to the species occupying the resource?; (3) does the presence of an additional uncolonised resource modify any effects?

4.2 MATERIALS AND METHODS

4.2.1 Fungal Isolates

Phanerochaete velutina, *Stereum hirsutum*, *Coriolus versicolor*, *Phlebia radiata* and *Vuilleminia comedens* were originally isolated from decaying beech wood from the Forest of Dean, UK, (National Grid Reference SO 611145). Laboratory stocks were routinely maintained and routinely subcultured as described in section 2.1- 2.2.

4.2.2 Experimental Design

Three 90 mm Petri dish bases were glued along a diagonal across the base of a 24 × 24cm Perspex tray (Fig.4.1). Within two of the Petri dish bases a 35mm Petri dish bases was glued centrally. Wood blocks precolonised by *P. velutina*, were added the central dish, as described in sections 3.2.1- 3.2.5, and after 23 d (Fig.4.1 and Fig.4.2) (Appendix IIa) an uncolonised resource or precolonised resource was added to one or both of the other dishes. Mycelial systems were photographed as described in section 2.9.1.

4.2.3 Phosphorus translocation and acquisition in Mycelial systems of

Phanerochaete velutina

³²P was supplied to 73 d old mycelial systems to mycelial systems encountering wood inocula colonised by a variety of fungi (Fig. 4.2). A stock solution of 20mM NH₄K₂PO₄

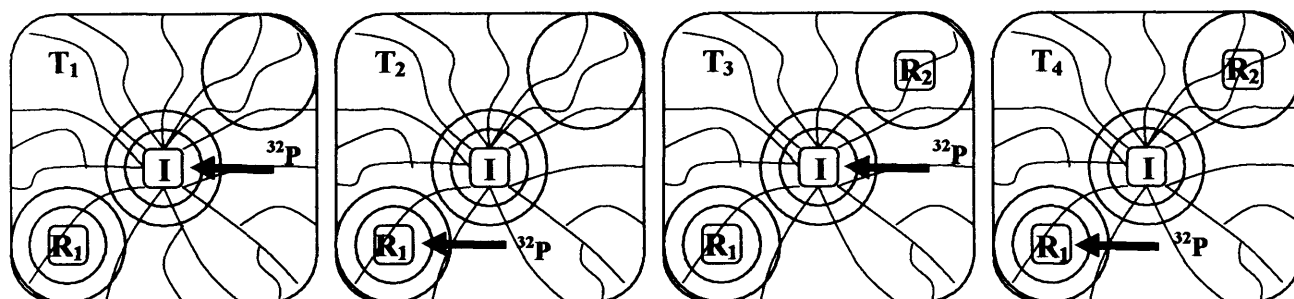


Figure 4.1 Diagram representing the experimental set up of soil systems inoculated with wood resources colonised by *P. velutina* (I). Four treatments were employed (T₁₋₄) where wood resources pre-colonised (R₁) or un-colonised (R₂) were added to the mycelial systems. Phosphorus labelled solution was added either around *P. velutina* inocula or around the pre-colonised resources. To the remaining soil, unlabelled phosphorus solution was added.

Figure 4.2

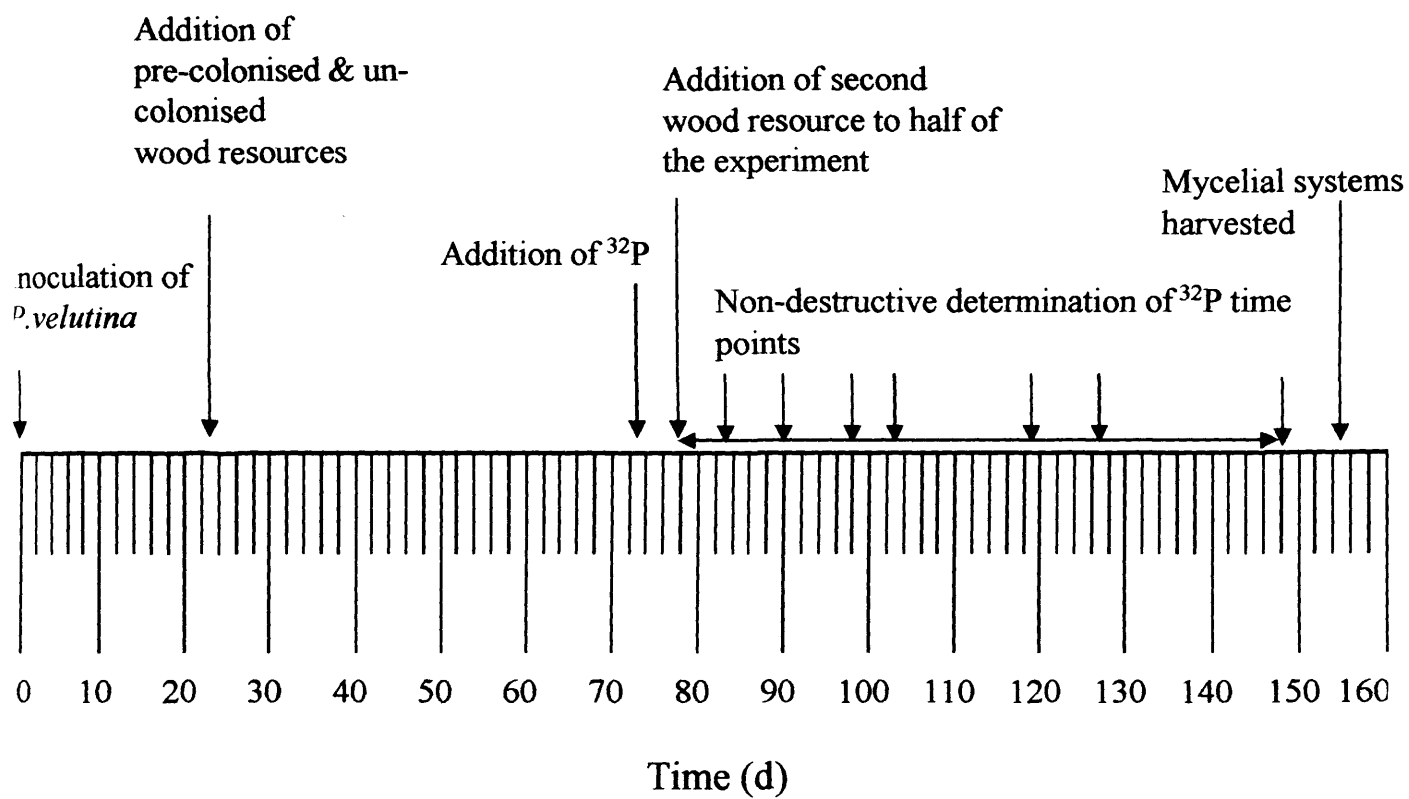


Figure 4.2 Time scale of the addition of ^{32}P to mycelial systems

(AnalaR) was made and to a volume of 50 ml 100 MBq ^{32}P orthophosphate (Amersham, carrier-free in dilute HCL) was added.

To 50 mycelial systems 8 x 50 μl aliquots of 20 mM $\text{NH}_4\text{K}_2\text{PO}_4$ (AnalaR) labelled with 0.8 M.Bq ^{32}P orthophosphate (Amersham, carrier-free in dilute HCL) were added uniformly to the 90mm diameter central compartment surrounding *P. velutina* colonised wood resources. To the remainder of the soil tray, 54 x 50 μl of unlabelled 20mM $\text{NH}_4\text{K}_2\text{PO}_4$ (AnalaR) was added uniformly through evenly spaced holes drilled through a Perspex 24cm² bioassay dish. To another 50 mycelial systems, labelled phosphorus solution was added uniformly to the soil contained within the 90mm radioisotope compartment surrounding the resource-restricted fungus (Fig.4.3). Controls were set up where systems of *P. velutina* contained a previously un-colonised resource. ^{32}P activity was monitored for a period of 70 days non-destructively in wood resources is described in section 2.8.6.

4.2.4 Effect of the addition of a previously uncolonised wood resource on phosphorus allocation in well established interacting mycelial systems.

A fresh previously uncolonised wood resource was added to 78 d old well established interacting mycelial systems of *Phanerochaete velutina*, 5 days after the addition of ^{32}P isotope. Phosphorus activity was monitored non-destructively with time.

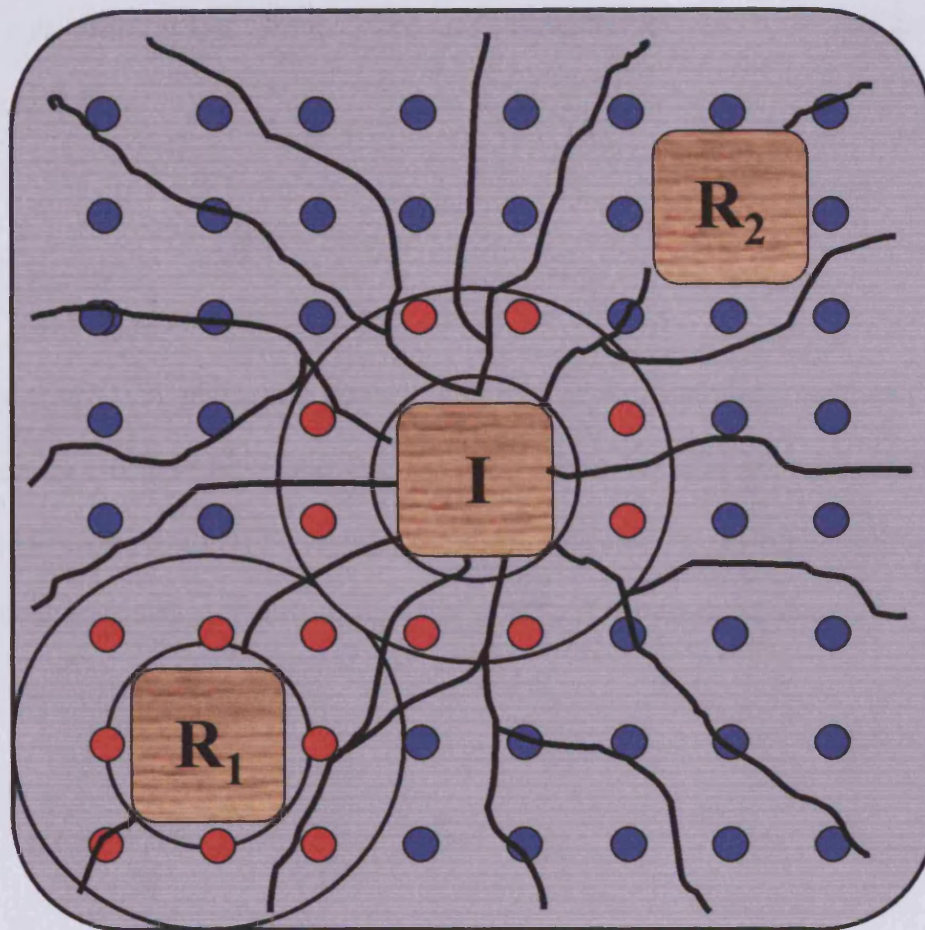


Figure 4.3 ^{32}P solution (●) was supplied to mycelial systems of *P. velutina* either to soil in close proximity to the inocula (I) or to the precolonised resource (R_1). A previous uncolonised wood resource (R_2) was added to half of the mycelial systems 5d after the addition of ^{32}P . Unlabelled phosphorus solution was added to the rest of the soil (●).

4.2.5 Destructive determination of ^{32}P

At 155d, mycelial systems were harvested. Destructive determination of ^{32}P activity in all inocula, precolonised resources and second uncolonised resources were determined (Sections 2.8.8).

4.2.6 Statistical analyses

One-way ANOVA, and where appropriate *priori* or *posteriori* tests were used to compare treatment means, following Levene's test for equality of variances. Kruskal-Wallis tests were employed to compare treatment medians with unequal variances. Statistical analyses were performed using MINTAB for windows version 13.

4.3 RESULTS

4.3.1 Mycelial morphology

Mycelial egression from wood inocula by *P. velutina* was evident at 3 d, (radial extension 0.05 cm d^{-1}) growth developed into well defined mycelial cords by 10 d and continued to extend until reaching the edge of the soil tray by 23 d.

4.3.1.1 *P. velutina* encountering the pre-coloniser *Coriolus versicolor*

In mycelial systems containing both one and two resources, fine regression of mycelia was apparent in systems at 43 d (Fig.4.4b, h, n, t). Extensive regression of mycelia occurred in the vicinity of the pre-coloniser at 155d (Fig.4.4r & x) and in systems containing two resources, similar outgrowth by *P. velutina* compared to that of the

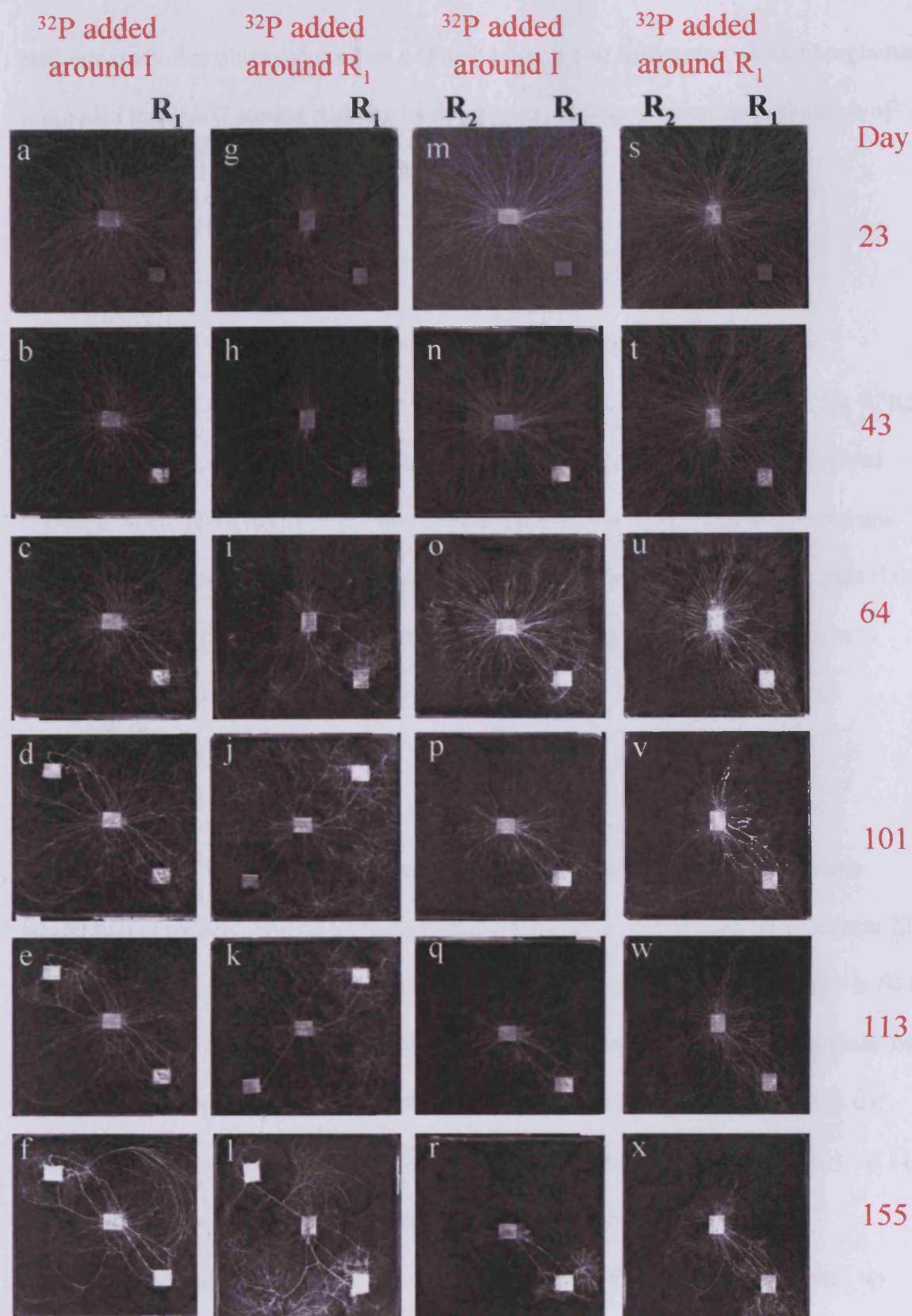


Figure 4.4 Digital images of mycelial systems of *P. velutina* encountering wood resources precolonised by *Coriolus versicolor* (R_1) and a second uncolonised resource (R_2). ^{32}P solution was added either in the vicinity of the inoculum (I) or the first new resource (R_1).

previous study was observed until 64 d (Fig 4.4m-o & s-u) In systems where phosphorus was added to the soil around both the inoculum and the precoloniser, re-exploration of mycelia was noted at 155 d (Fig 4.4f & l).

4.3.1.2 *P. velutina* encountering the pre-coloniser *Stereum hirsutum*

Fine regression of mycelia occurred at 43 d (Fig 4.5a,g, m, s) through to 64 d (Fig 4.5b,h, n, t). Re-exploration of mycelia was observed in systems containing the precolonised resource, where phosphorus was added around the inoculum (Fig 4.5p) and in systems containing two resources where phosphorus was supplied around *Stereum hirsutum* (Fig 4.5j). Thickening of mycelial cords was evident contacting the precolonised resource (R₁) particularly at 155d in systems only containing the precoloniser (Fig 4.5r,x).

4.3.1.3 *P. velutina* encountering the pre-coloniser *Vuilleminia comedens*

Regression of mycelia around the margin of the systems occurred in all treatments at 23 d (Fig.4.6 a, g, m, s) until 43d (Fig.4.6b, h, n, t). Thickening of cords was evident at 64 d (Fig.4.6c,i,o,u) in the area containing the precoloniser. Outgrowth occurred out from the second resource at 101 d in systems where phosphorus was supplied to the soil in the vicinity of the precolonised resource containing *Vuilleminia comedens* (Fig.4.6v). At 113 d re-exploration continued from the second resource (R₂) in treatments where phosphorus was added around the precolonised resource (Fig.4.6w), this persisted up

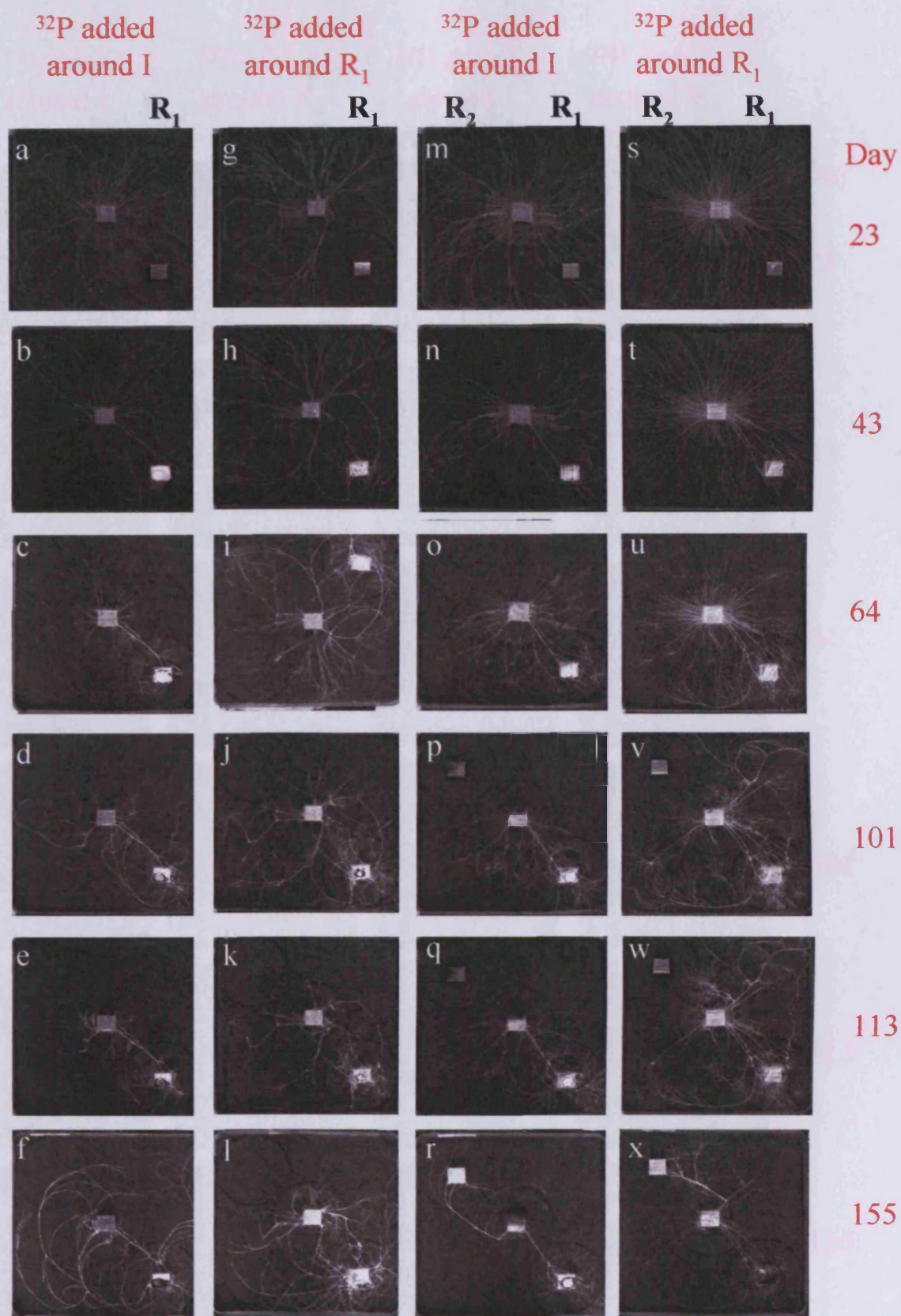


Figure 4.5 Digital images of mycelial systems of *P. velutina* encountering wood resources precolonised by *Stereum hirsutum* (R_1) and a second uncolonised resource (R_2). ^{32}P solution was added either in the vicinity of the inoculum (I) or the first new resource (R_1).

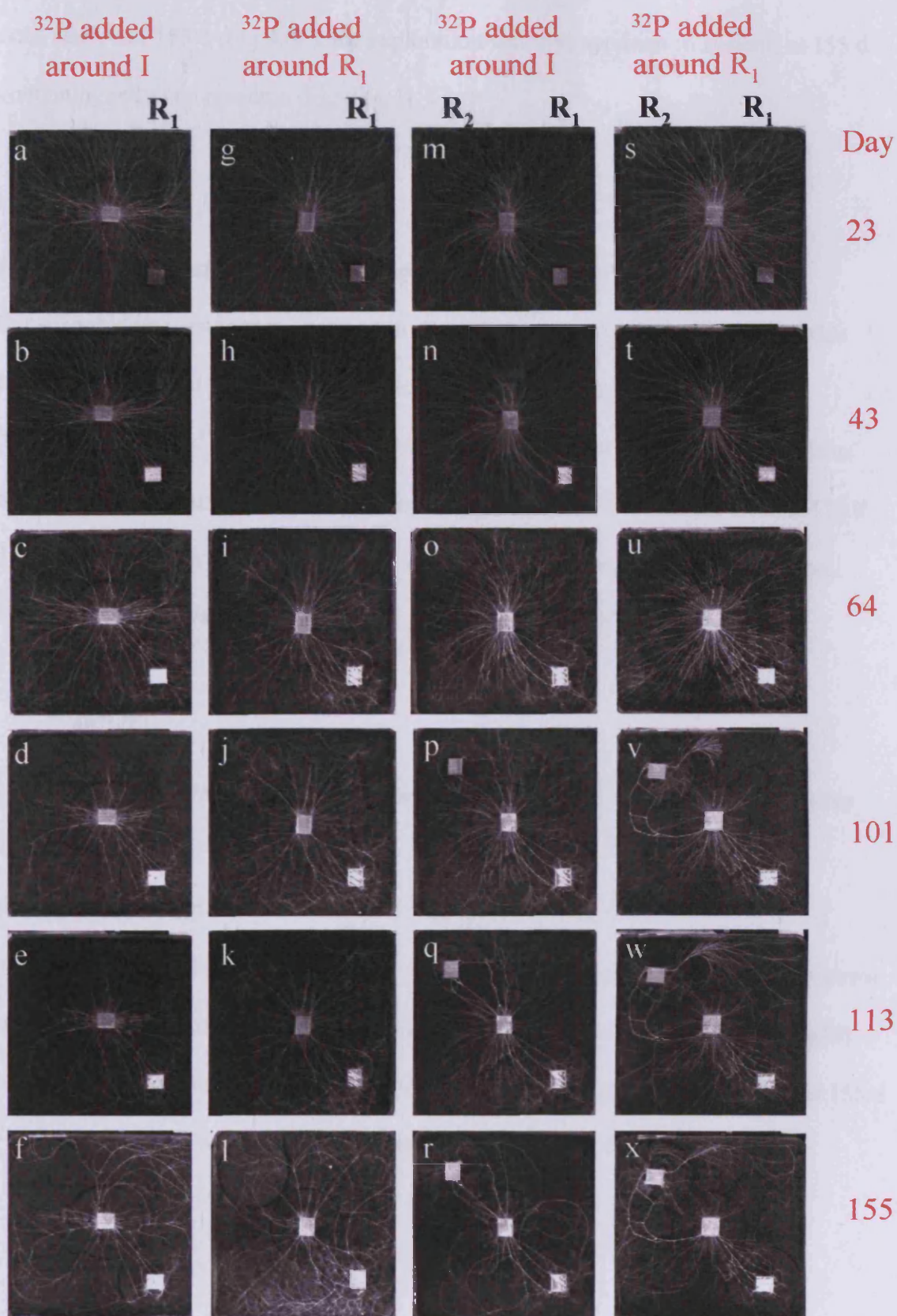


Figure 4.6 Digital images of mycelial systems of *P. velutina* encountering wood resources precolonised by *Vuilleminia comedens* (R_1) and a second uncolonised resource (R_2). ^{32}P solution was added either in the vicinity of the inoculum (I) or the first new resource (R_1).

until harvest at 155 d (Fig 4.6x). Re-exploration was also apparent in systems at 155 d containing only one resource (Fig.4.6f, l).

4.3.1.4 *P. velutina* encountering the pre-coloniser *Phlebia radiata*

Considerable regression was observed in all treatments at 64 d (Fig.4.7c, I, o, u) this continued until 113d in systems containing only the precolonised wood resource (Fig.4.7d, j, e, k) and re-exploration was evident at 155d (Fig.4.7f, l, r, x). In systems containing two resources, thickening of cords to the second resource occurred at 113 d (Fig.4.7p,u), and regression was evident in the vicinity of the precolonised resource where phosphorus was added around the inoculum (Fig 4.7 r).

4.3.1.5 *P. velutina* encountering a previously un-colonised wood resource (positive controls)

Mycelial regression in all treatments persisted until 64d (Fig.4.8c, i, o, u), At 155 d extensive re-exploration by *P. velutina* occurred towards the second resource in systems containing two resources where phosphorus was added around the inoculum (Fig.4.8r) and outgrowth from the first resource added to the mycelial systems was evident at 155 d (Fig.4.8l) in systems where phosphorus was supplied around that resource.

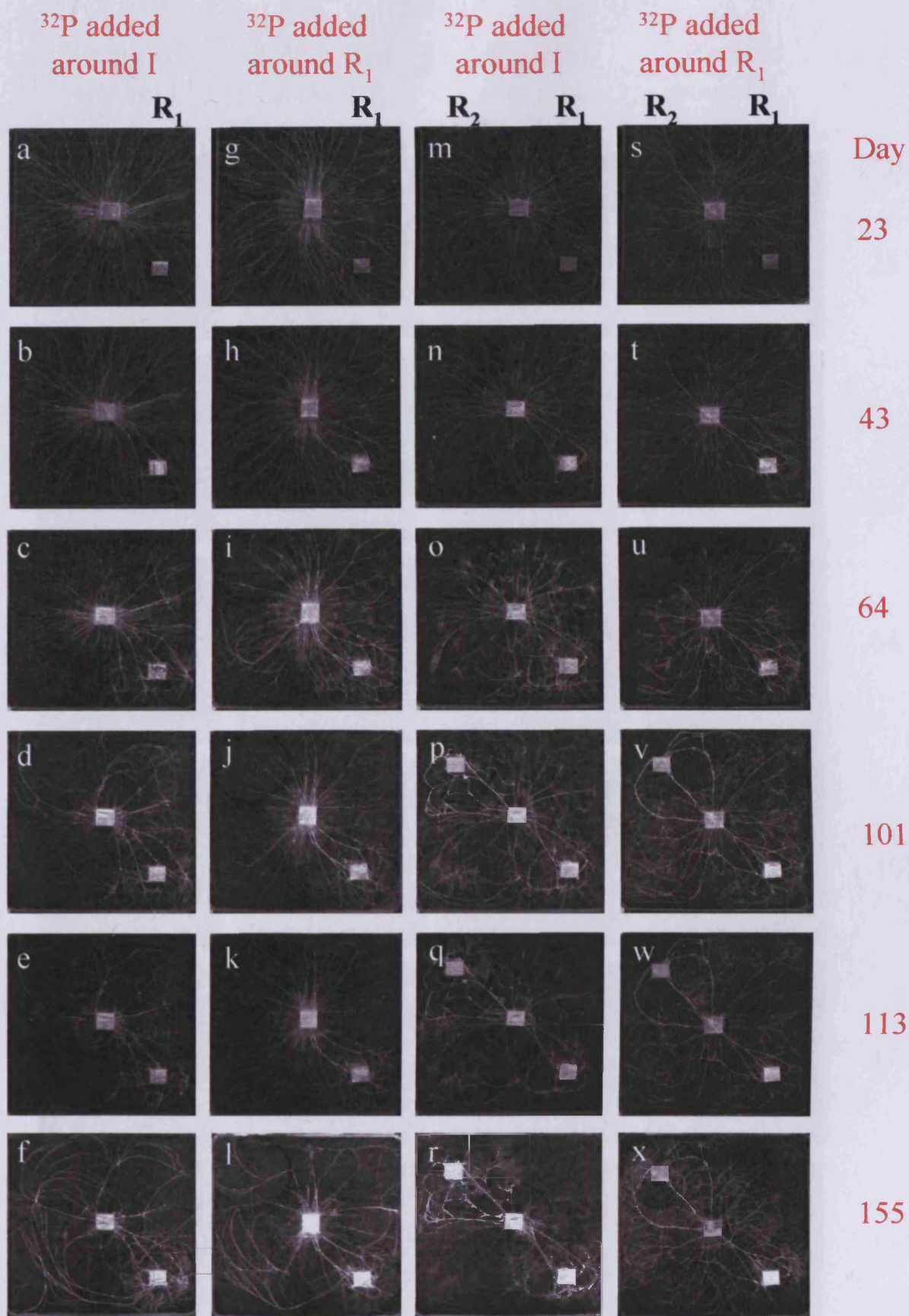


Figure 4.7 Digital images of mycelial systems of *P. velutina* encountering wood resources precolonised by *P. radiata* (R_1) and a second uncolonised resource (R_2). ^{32}P solution was added either in the vicinity of the inoculum (I) or the first new resource (R_1).

4.3.2 *Phosphorus acquisition and translocation*

4.3.2.1 *Phosphorus acquisition and translocation in mycelial systems with one new resource*

Rapid uptake of ^{32}P from soil in treatments where ^{32}P solution was supplied to the area in the vicinity of the inoculum or precolonised wood resource was evident in mycelial systems encountering one new resource was added but not when two were added (Fig 4.9 a, d), phosphorus translocation by *P. velutina* to these resources (uncolonised or precolonised) was detectable when first monitored non destructively 5d after the addition of ^{32}P . Phosphorus levels increased in mycelial systems with time (Fig 4.9a).

Significant differences between treatments were detected ($P < 0.05$), and this depended on what the precoloniser of that new resource was. Initially, most uptake to the *P. velutina* inoculum block was when *P. radiata* was the precoloniser of the new resource, and least uptake was with *V. comedens* as precoloniser (Fig. 4.9a).

Phosphorus uptake and translocation in mycelial systems of *P. velutina* interacting with the wood precoloniser *Coriolus versicolor* was significantly greater than in systems of *P. velutina* interacting with other wood decay fungi (Fig 4.9 c, d). Activity remained constant in these inocula over time, except for systems with *P. radiata* and *S. hirsutum*, where a significant ($P < 0.05$) increase in activity was evident (Fig. 4.9a).

In treatments where ^{32}P was added to the soil in the vicinity of the new resource, precolonised or uncolonised, ^{32}P was detected in the new resource when first

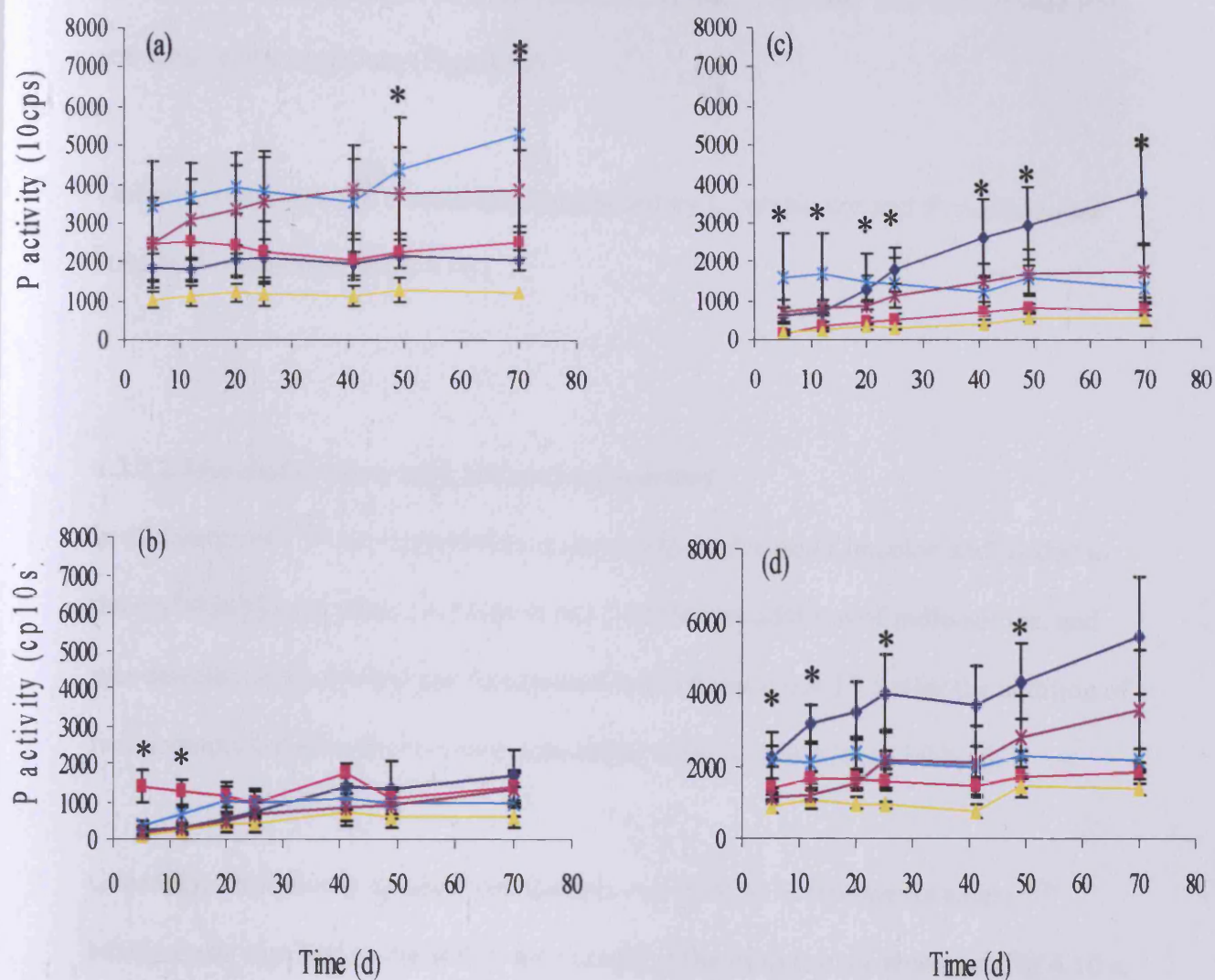


Figure 4.9 The time and course of allocation of soil-derived ^{32}P in *P. velutina* inoculum wood blocks (a, c) and wood pre-colonised by other decay fungi (b, d), in systems to which isotope was added to soil near the *P. velutina* inoculum (a, b) or near the resource pre-colonised by other fungi (c, d). Radioactivity was monitored non-destructively from above. One-way ANOVA and S tests indicated significant differences (*, $P < 0.05$) Error bars are SEM. Symbols: *Coriolus versicolor* (◆), *Vuilleminia comedens* (▲), *Phlebia radiata* (×) and *Stereum hirsutum* (*). Positive control systems were supplied with a fresh previously un-colonised beech wood resource (■).

monitored (fig 4.9d), some was translocated back to the inoculum (Fig 4.9c), though the amount in inocula derived from precolonised baits was only half that derived from soil local to the inoculum (Figs 4.9a)

Greatest uptake was by resources precolonised by *C. versicolor* and *P. radiata*, and least by *V. comedens* (Figs 4.9d)

4.3.2.2 Mycelial systems with two resources added

In all treatments ^{32}P was detected in resources (precolonised / uncolonised) added to the mycelial system when first monitored 5 d after the addition of radioisotope, and was detected in the second previously uncolonised resources 12 d after the addition of radioisotope, 6 d after the resource was added to the systems (Fig4.10 b, c).

Generally, Phosphorus uptake from the soil was greatest in treatments where ^{32}P solution was supplied to the soil in the vicinity of the inoculum or resource (Fig 4.10 a, e).

^{32}P translocation by *P. velutina* to the second previously uncolonised resource was similar in treatments where ^{32}P was added either to the soil in the vicinity of the inoculum or to the precolonised resource (Fig 4.10c,f).

In systems where ^{32}P was added in close proximity to the inoculum, wood resources precolonised by *V. comedens* commonly had the lowest counts of P activity (Fig 4.10 a, b, c).

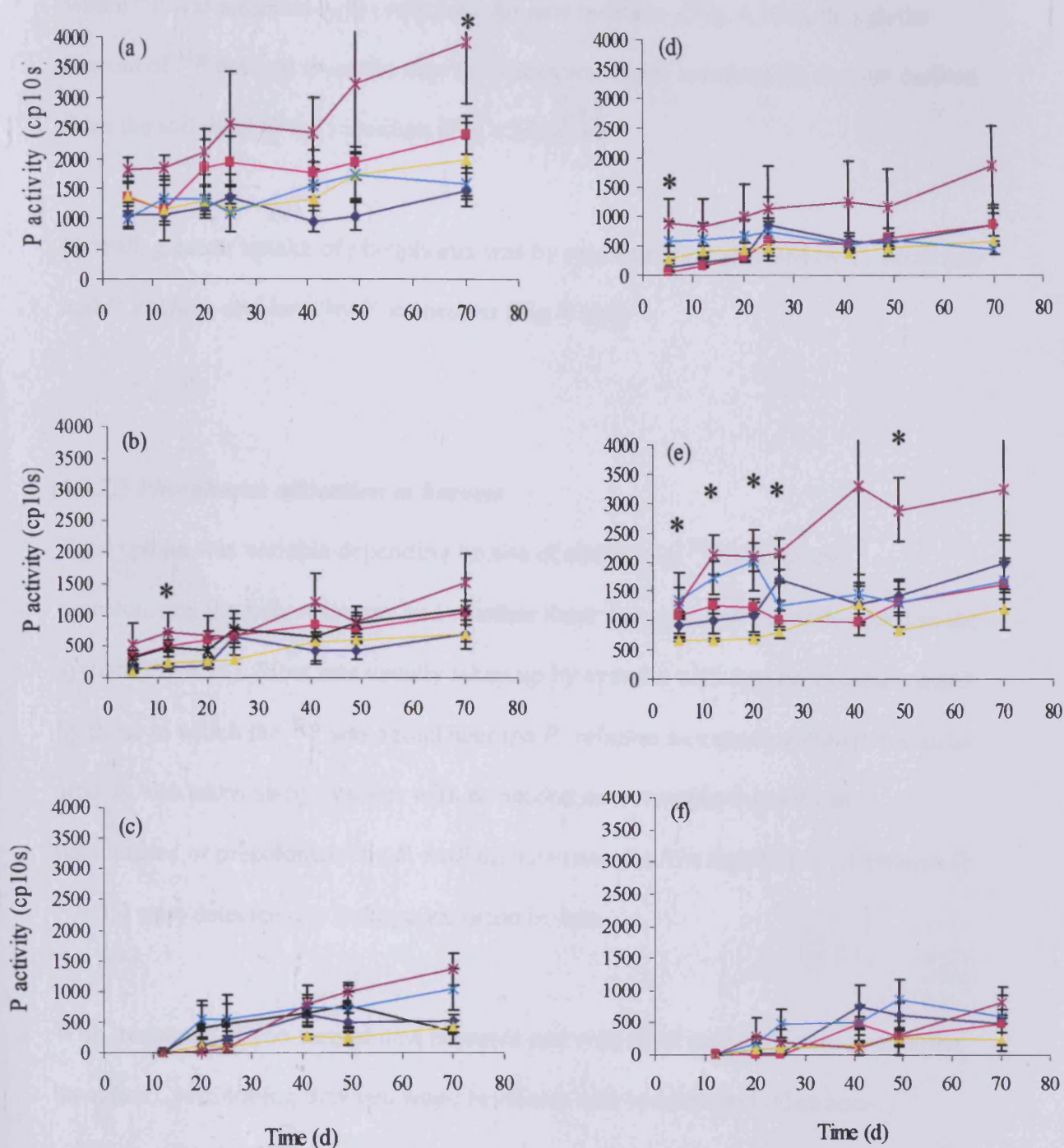


Figure 4.10 The time and course of allocation of soil-derived ^{32}P in *P. velutina* inoculum wood blocks (a, d) wood pre-colonised by other decay fungi (b, e) and previously uncolonised wood (c, f), in systems to which isotope was added to soil near the *P. velutina* inoculum (a, b, c) or near the resource pre-colonised by other fungi (d, e, f). Radioactivity was monitored non-destructively from above. * denotes significant difference ($P < 0.05$) between means, following One-way ANOVA and Tukey's pairwise comparisons. Symbols: *Coriolus versicolor* (◆), *Vuilleminia comedens* (▲), *Phlebia radiata* (×) and *Stereum hirsutum* (*). Positive control systems were supplied with a fresh previously un-colonised beech wood resource (■).

Phosphorus uptake and transportation back to the inocula was evident in systems where ^{32}P was supplied to the soil near the new resource (Fig. 4.10d), though the amount of ^{32}P derived from the new resources was much less than the amount derived from the soil local to the inoculum (Fig 4.10a).

Overall, greatest uptake of phosphorus was by resources precolonised by *C. versicolor* and *P. radiata*, and least by *V. comedens* (Fig 4.10e).

4.3.2.3 Phosphorus allocation at harvest

Total uptake was variable depending on site of addition of ^{32}P , the species precolonising the new resource, and whether there was a second resource added to the system (Fig.4.11). Most was usually taken up by systems with two new resources and by those to which the ^{32}P was added near the *P. velutina* inoculum, although a similar amount was taken up by systems with no second new resource but with an uncolonised or precolonised by *P. radiata* new resource. No significant differences ($P > 0.05$) were detected due to large variation in data.

With treatment T1 (no second new resource and with label added close to *P. velutina* inoculum), partitioning between wood resources was variable and often non-significant ($P > 0.05$); relatively more activity was detected in the *P. velutina* inoculum than in the precolonised *P. radiata* resource, whereas there was greater activity in the precolonised *C. versicolor* resource than in the *P. velutina* inoculum.

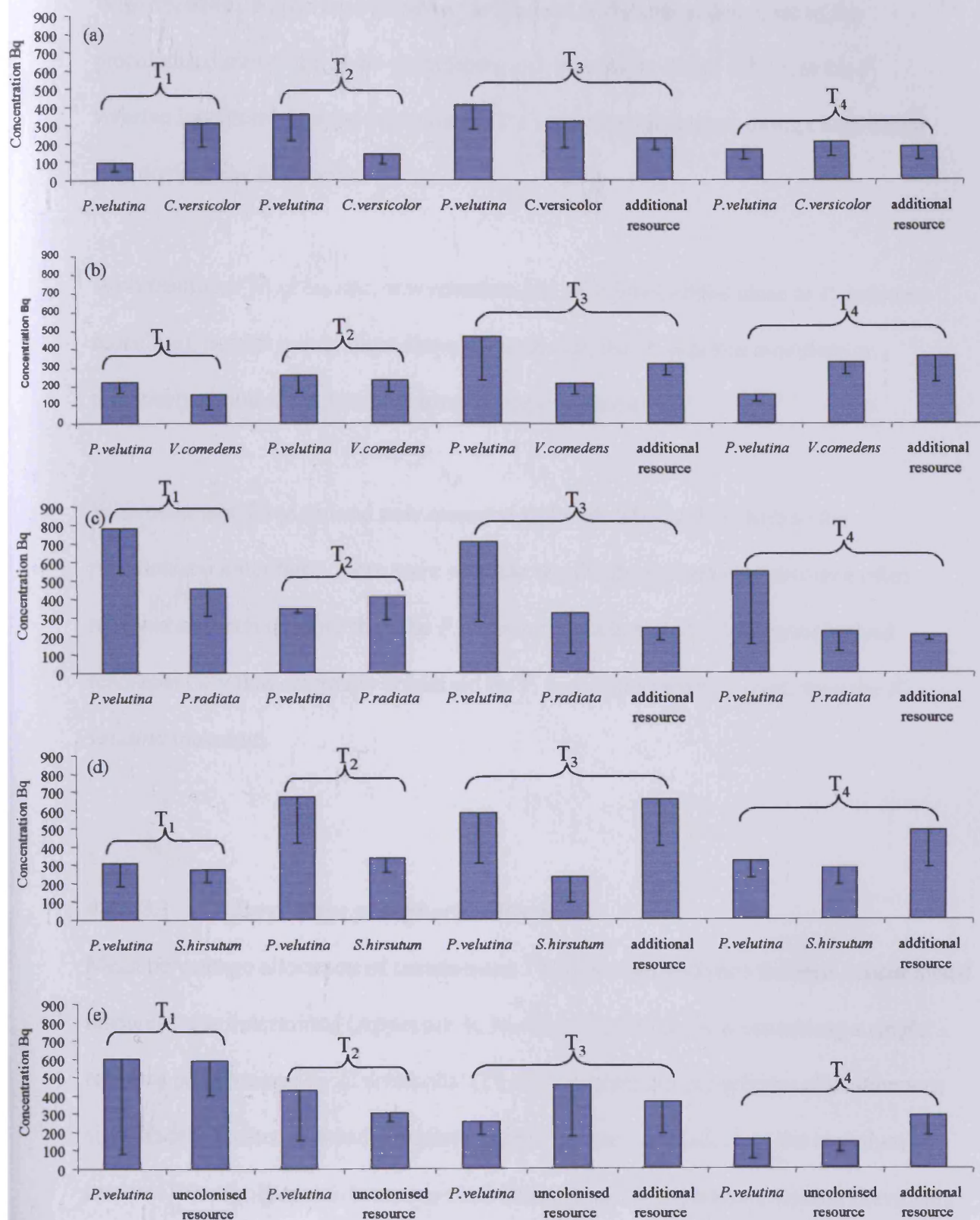


Figure 4.11 Total ^{32}P uptake in *P. velutina* inoculum, wood pre-colonised by *C. versicolor* (a), *V. comedens* (b), *P. radiata* (c) and *S. hirsutum* (d) and un-colonised (e) and a previously un-colonised fresh wood resource 82 days after isotope addition to soil near the inoculum (treatment 1 & 3) or the pre-colonised resource (treatment 2 & 4)

With treatment T2 (no second new resource and with label added close to the precolonised inoculum), with precolonised *C. versicolor* and *S. hirsutum* the *P. velutina* inoculum received more than the precolonised resource, though with others partitioning was fairly even.

With treatment T3 (a second new resource and with label added close to *P. velutina* inoculum), no real trends were discernible, though the *P. velutina* inoculum or previously uncolonised resource usually acquired most ^{32}P .

With treatment T4 (a second new resource and with label added close to the precolonised inoculum), there were no clear trends; the second new resource often received as much or more than the *P. velutina* inoculum, and of the precolonised resources only that originally colonised by *V. comedens* received more than the *P. velutina* inoculum.

4.3.2.3.1 *Percentage phosphorus allocation*

Mean percentage allocation of translocated ^{32}P to inocula and precolonised / uncolonised resources was determined (Appendix II, b). Generally in systems containing a single resource precolonised by *C.versicolor* (T1, T2), percentage phosphorus allocation was significantly greater in wood resources where ^{32}P was added close to the inoculum or resource. No significant differences were detected in T3-T4, where a second previously uncolonised resource was added 5 d after the addition of the radiotracer.

Regardless of experimental design (T1 – T4), no significant differences in percentage phosphorus allocation in mycelial systems containing the precoloniser *V.comedens* was detected in *P.velutina* inocula, precolonised and uncolonised resources in systems (Appendix IIb).

Mycelial systems containing the precoloniser *P. radiata*, phosphorus allocation was significantly higher ($P<0.05$) in *P. velutina* inocula where ^{32}P was added nearby (T1). There was no significant differences in phosphorus allocation to wood resources within the mycelial systems for T2-T4 ($P<0.05$), however, phosphorus allocation to *P.velutina* in T4, was significantly lower than *P.velutina* inocula for T1 ($P<0.05$) (Appendix IIb).

Mycelial systems containing wood resources precolonised by *Stereum hirsutum*, no significant difference were detected between wood resources precolonised / uncolonised and *P.velutina* inoculum in all treatments, No significant differences were detected in *P.velutina* inocula, resources precolonised/uncolonised in relation to treatment design (Appendix IIb).

Percentage phosphorus allocation to control systems, where a previously uncolonised wood resource was added to the system (r1), followed by another uncolonised resource (r2), added to the system 5 days after the addition of ^{32}P , was significantly lower ($P<0.05$) in the first resource than the second resource (T3). Allocation to the first resource was significantly lower in T4 than T1 ($P<0.05$) (Appendix IIb).

4.4 DISCUSSION

This study has shown for the first time that when a resource arriving on an established system is already colonised, the presence of a fungus can have major effects on uptake and allocation of phosphorus by mycelial systems of *P. velutina* are affected. These effects are specific to the species occupying the resource.

In mycelial systems containing only one resource (precolonised / uncolonised), mycelial morphology was generally not dissimilar to that observed in chapter 3. However, In some treatments, greater regression of the mycelium occurred in the vicinity of the precoloniser, particularly resources precolonised by *C. versicolor* and *P. radiata*, greater re-exploration of soil by mycelia was observed in treatments precolonised by *V. comedens* and earlier re-exploration of mycelia occurred in treatments precolonised by *S. hirsutum*. These differences detected in foraging patterns exhibited may be due to the uniform addition of phosphorus to the soil.

There was no evidence of mycelial patches which, in one of the few other studies to examine effects of addition of new resources to established systems, formed system-wide but predominantly in the part with the new resource (Wells, Donnelly & Boddy, 1997). There was, however, a major difference in the nutrient status of the soils used in the two studies; mycelial patches developed on a soil that was considerably poorer in nutrients (due to being mixed with an equal quantity of sand). Lack of development of mycelial patches in the present study confirms further the assertion in the earlier study that these patches develop to obtain nutrients to enable colonisation of the newly added resource.

The difference in combative ability of the fungus colonising the new resource seems also to affect uptake and translocation of ^{32}P (as revealed by non-destructive monitoring over time). Initially most uptake of ^{32}P in the vicinity of the *P. velutina* inoculum block was when *P. radiata* was the precoloniser of the new resource, and least was with *V. comedens* as precoloniser, presumably reflecting the greater need for phosphorus during combat against an aggressive opponent compared with a weak opponent. The demand for phosphorus during combat against aggressive antagonists is further emphasised by the fact that ^{32}P was translocated to the new resources, but those containing *V. comedens* commonly had the lowest counts initially. This demand was not only met by ^{32}P taken up near the inoculum, but also by uptake close to the new resource, where again initially least was supplied to wood precolonised by *V. comedens*, and most supplied to wood precolonised *C. versicolor* and *P. radiata*. Uptake of ^{32}P from a local supply was clearly as important as translocation from elsewhere, though this was not quite as dramatic as in a previous study (Wells, Boddy & Donnelly, 1998), where rate of uptake by a previously uncolonised resource was two orders of magnitude greater than from a distal supply.

As reported previously (Boddy L, 1999; Cairney, 1992; Lindahl, Finlay, Olsson, 2001; Wells, Harris & Boddy, 1998b) there is bidirectional translocation within systems. In the present study there was ^{32}P movement both from and to the *P. velutina* inoculum block in gradient free systems. Again, as in a previous study (Hughes, & Boddy, 1994), ^{32}P acquired near a new previously uncolonised resource was translocated not only into that new resource but also back to the original inoculum and beyond to a further resource, where present, accumulating more than in the inoculum wood block. Moreover, movement to the second additional resource also occurred in

the present study when the other newly arrived resources were precolonised by wood decay fungi.

The results at final harvest were very variable, and there was no trend of dramatically more ^{32}P accumulation in any specific type of resource. Previous studies with multiple resources added to established systems revealed complex patterns of ^{32}P allocation to different resources reflecting size, time of colonisation, carbon availability and presumably demand (Hughes & Boddy, 1994; Wells, Harris & Boddy, 1998a; Wells, Harris & Boddy, 1998b). Neither in the present experiment, nor in previous ones using similar systems, is there evidence that demand is the major driving force for translocation. Previously we have suggested that 'observed' (i.e. accumulation / loss at a particular site) translocation is not an absolute measure, but rather indicates the extent to which P is loaded from a translocation stream in regions where it is being actively utilised or stored (Wells, Harris & Boddy, 1998a; Wells, Harris & Boddy, 1998b). The translocation stream was analogised to the flow of an alternating current from which power is only consumed when the user plugs into the mains. How the transport system develops and alters is currently being explored using photon counting scintillation imaging (PCSI), to image amino acid translocation in continuously in foraging mycelium of *P. velutina* (Tlalka et al., 2002; Tlalka et al., 2003).

In conclusion, the presence of a fungus occupying a resource arriving on an established mycelial system in soil clearly has major effects on uptake and allocation of phosphorus.

These effects are specific to the species occupying the resource, and probably also to the species forming the established mycelium on soil.

CHAPTER 5

Phosphorus translocation by *Phanerochaete velutina* encountering unit restricted fungi on soil: Effect of Decay State

5.1 INTRODUCTION

The arrival of woody resources with different quality in relation to the presence of other wood decay fungi within the resource has been shown to have a major effect on the uptake and translocation of ^{32}P in mycelial systems of *P. velutina* (Chapter 4). However, nutrient studies on mycelial interactions between resource restricted fungi at different states of decay have been limited.

Boddy & Abdalla (1998) reported that qualitative studies on mycelial systems of *P. velutina* was affected by both the state of decay and the presence of simultaneous encounter of other wood decay fungi precolonising the resource. Mycelial systems of *P. velutina* encountered wood resources precolonised by *S. hirsutum*, *S. rugosum* and *U. deusta*. Preferential colonisation of wood resources was evident where previously uncolonised wood resources were colonised greater in turn than 6 week old precolonised resources and further by 2 year old colonised resources. *P. velutina* preferentially colonised the resources in that order regardless of the precoloniser inhabiting that specific resource.

This chapter aims to elucidate a further understanding of phosphorus translocation to resources supplied behind the growing mycelia in relation to age of precolonies

resources and whether; (1) the presence of other wood decay fungi and state of decay of wood resources can affect phosphorus translocation by *P. velutina* to these wood resources? (2) Does the presence of one or two resources, differing in resource quality and state of decay affect the outcome of interactions in mycelial systems?

5.2 MATERIALS AND METHODS

5.2.1 *Fungal isolates*

Phanerochaete velutina, *Stereum hirsutum* and *Vuilleminia comedens* were originally isolated from decaying beech wood from the Forest of Dean, UK, (National Grid Reference SO 611145). Laboratory stocks were routinely maintained and routinely subcultured as described in section 2.1- 2.2.

5.2.2 *Preparation of inocula and precolonised wood resources of different states of decay*

Beech wood blocks 20×20×10 mm³ were cut, stored and autoclaved as described in section 2.5. Batches of 25, twice autoclaved wood blocks at 24 hr intervals, were transferred aseptically into 14cm diameter Petri dishes containing either two week old cultures; *P. velutina*, *S. hirsutum* or *V. comedens* grown on MA. The dishes were then incubated at 20 °C for 8 weeks to ensure colonisation of the wood. Also, wood blocks of 3 year old precolonised *S. hirsutum* and *V. comedens* using the method described above but incubating in 2l wide necked conical flasks containing 500ml of MA.

5.2.3 Preparation of mycelial systems

Petri dish bases (5cm diameter) were glued centrally inside 24 × 24cm Perspex trays (Fig.5.1). The former acted as radioisotope compartments. Non sterile soil was collected and prepared as described in section 2.8.1-2.8.2.

Eight week old *Phanerochaete velutina* inocula were removed from the 14cm dishes, and were prepared for the addition onto compressed soil as described in section 2.8.3. *P. velutina* inocula were placed firmly into a central position contained within the 5cm radioisotope compartment. At 31 d, when mycelial systems reached the edge of the soil dish, uncolonised, or precolonised resources of two decay ages, were placed onto the mycelial system, behind the mycelial margin (see table 5.1 for experimental pairings). Sub-samples of inocula, uncolonised wood and precolonised resources were taken to determine Relative Density (g cm^{-3}). Inoculated trays were incubated at 20°C as described in section 2.8.4.



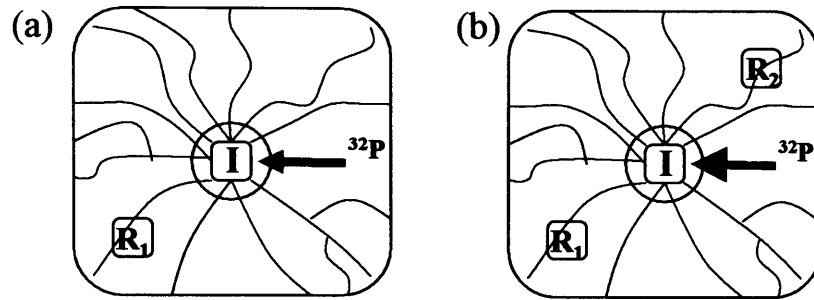


Figure 5.1 Diagram representing the experimental set up of soil systems inoculated with wood resources colonised by *P. velutina* (I). Two designs were employed (a & b) where wood resources pre-colonised (R₁) or uncolonised / precolonised (R₂) ; see table 5.1 for experimental pairings, were added to the mycelial systems. Phosphorus labelled solution was added to the soil around *P. velutina* inocula. To the remaining soil, unlabelled phosphorus solution was added.

Treatment	Resource 1	Inocula	Resource 2
1	Sh 3yrs	Pv	
2	Sh 8wks	Pv	
3	Vc 3yrs	Pv	
4	Vc 8wks	Pv	
5	Sh 3yrs	Pv	Vc 8wks
6	Sh 3yrs	Pv	Sh 8wks
7	Vc 3yrs	Pv	Vc 8wks
8	Vc 3yrs	Pv	Sh 8wks
9	Vc 3yrs	Pv	Wb
10	Sh 3yrs	Pv	Wb
11	Vc 8wks	Pv	Wb
12	Sh 8wks	Pv	Wb

Table 5.1 Experimental pairings to determine the effect of decay state of resources on phosphorus translocation and outcome of interaction. Where inocula of *P. velutina* 4cm³ (Pv) encountered combinations of *Stereum hirsutum* (Sh), *Vuilleminia comedens* (Vc).

5.2.4 Addition of ³²P to mycelial systems

To 33 d old mycelial systems of *P. velutina*, two days after the addition of uncolonised / precolonised resources, ³²P solution was added to 1/16th of the soil for each mycelial system (Fig.5.2)

A stock solution of 20mM NH₄K₂PO₄ (AnalaR) was made to a volume of 1 litre. 100 ml of stock solution contained 159 M.Bq ³²[P]orthophosphate (Amersham, carrier-free in dilute HCL) 4 x 50µl aliquots of the labeled solution was supplied uniformly to the soil within the 5cm diameter radioisotope compartment, 0.32 M.Bq ³²[P]orthophosphate / tray. To the remainder of the soil tray, 60 x 50µl of unlabeled 20mM NH₄K₂PO₄

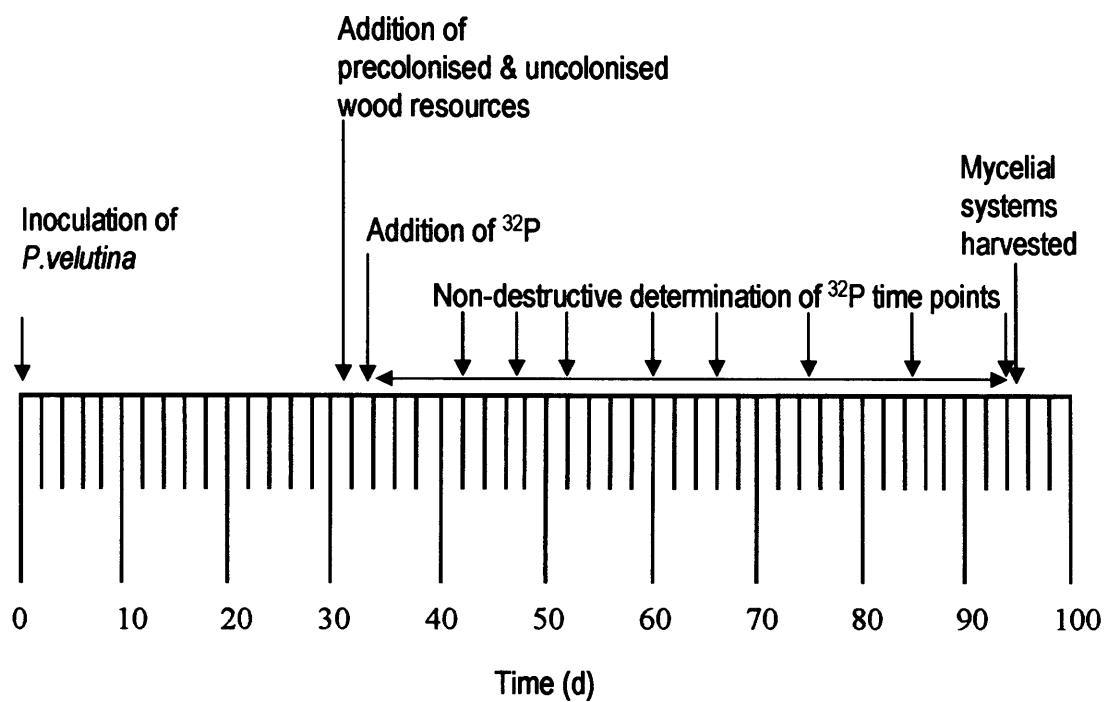


Figure 5.2 Time scale of the addition of ^{32}P to mycelial systems

(AnalaR) was added uniformly through evenly spaced holes drilled through a Perspex 24cm² bioassay lid.

Non-destructive determination of phosphorus activity in wood resources was determined as described in section 2.8.6. Phosphorus activity was monitored for a period of 59 days.

5.2.5 Effect of the addition of a single pre-colonised wood resource of different decay states on phosphorus translocation in mycelial cord systems

At 33 d, single 8 week, or three year old precolonised wood resources of *Stereum hirsutum* or *Vuilleminia comedens* were positioned directly onto the colony on a diagonal from *P. velutina* inocula, 2 cm away from the edge of the tray. In control systems, a fresh previously uncolonised wood resource was placed onto the mycelial system. Five replicates were made of each treatment. The trays were kept in polythene bags to minimise moisture loss and were incubated in the dark at 20 °C. 5 replicates were set up for each treatment.

5.2.6 Effect of the addition of two precolonised wood resources of different decay states on phosphorus translocation in mycelial cord systems

Two wood resources at different states of decay, uncolonised or precolonised by *Stereum hirsutum* or *Vuilleminia comedens* were added to 31d old mycelial systems of *P. velutina*. These were positioned directly onto the colony at opposite ends on a diagonal from the *P. velutina* inoculum, 2cm away from the edge of the soil tray. The trays were

kept in polythene bags to minimise moisture loss and were incubated in the dark at 20°C.

5 replicates were set up for each treatment.

5.2.7 Decay rate and colonisation of beech wood resources

Before the start of the experiments the initial relative density (RD g cm⁻³) of inocula, uncolonised and precolonised resource resources was obtained. At 95 days mycelial systems were harvested and the final RD was determined to estimate wood decay rates (g mg³d⁻¹); section 2.7 (Table 5.2).

Wood chips from a sub-sample of pre-colonised wood resources and previously uncolonised resources were plated aseptically onto 2% Malt Extract Agar as described in section 2.10 to determine outcome of mycelial interactions in wood.

5.2.8 Statistical analyses

One-way ANOVA and *posteriori* tests were used to compare treatment means, following Levene's test for equality of variances. Kruskal-Wallis tests were employed to compare treatment medians with unequal variances. Paired t tests were performed when comparing ³²P activity translocated by *P. velutina* containing two wood resources.

Wilcoxon's signed rank test was employed to compare treatment medians with unequal variances. Statistical analyses were performed using MINTAB for windows version 13.

Wood resource un-colonised or colonised by:	Relative Density at start of experiment (g cm ⁻³)
<i>P. velutina</i>	0.572
<i>S. hirsutum</i> 8wks	0.589
<i>S. hirsutum</i> 3yrs	0.358
<i>V. comedens</i> 8wks	0.569
<i>V. comedens</i> 3yrs	0.403
un-colonised	0.605

Table 5.2 Relative densities (g cm⁻³) of wood resources, prior inoculation of soil trays of *Phanerochaete velutina* inocula, precolonised resources (for 8 weeks and 3 years) and un-colonised wood blocks.

5.3 RESULTS

5.3.1 *Phosphorus acquisition in mycelial systems containing one new resource*

Generally, ^{32}P translocation to wood resources precolonised for 3 years by *Vuilleminia comedens* and *Stereum hirsutum* was initially high, activity gradually decreased with time (Fig.5.3). Initial translocation of ^{32}P by *P. velutina* from soil was significantly higher ($P < 0.05$) in systems containing three year old precolonised resources by *V. comedens* than in resources precolonised by *S. hirsutum* for 8 weeks (Fig.5.3). An increase in phosphorus activity at 12 and 17d was detected in systems containing 8 week old resources colonised by *V. comedens*, however this was not detected significantly ($P > 0.05$).

5.3.2 *Phosphorus acquisition in mycelial systems of P. velutina containing two new resources*

^{32}P translocation to wood resources R1 in T5-T8 (mycelial systems containing resources R1; precolonised for 3 years by *V. comedens* or *S. hirsutum*, and R2; precolonised for 8 weeks by *V. comedens* or *S. hirsutum*) were initially high and declined gradually with time (Fig.5.4a). No significant differences at a particular time were detected between these resources; T5 – T8 ($P > 0.05$). Generally ^{32}P activity was greater (but not significant) in wood resources precolonised for 3 years by *V. comedens* and *S. hirsutum* where the second resource contained the 8 week old precoloniser *S. hirsutum* (T8, T6), than resources precolonised for 3 years by

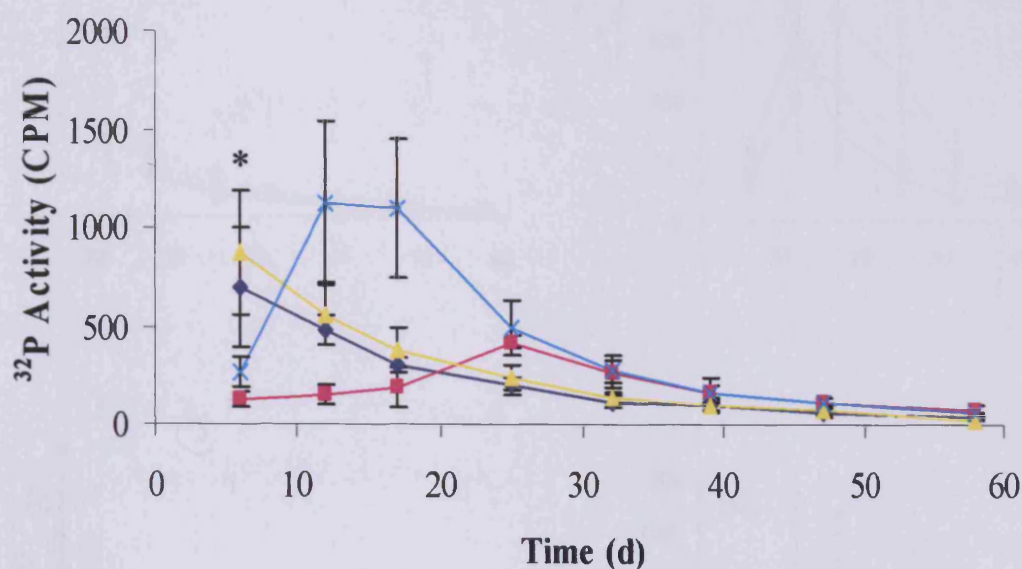


Figure 5.3 The time and course of allocation of soil-derived ^{32}P by *P. velutina* in systems containing a single wood resource. Wood resources (treatments 1-4) were either precolonised for 8 weeks by *Stereum hirsutum* (■) and *Vuilleminia comedens* (×), or for 3 years by *S. hirsutum* (◆) and *V. comedens* (▲). Radioactivity was monitored non-destructively. * denotes significant difference ($P \leq 0.05$) between means, following One-way ANOVA and Tukey's pairwise comparisons.

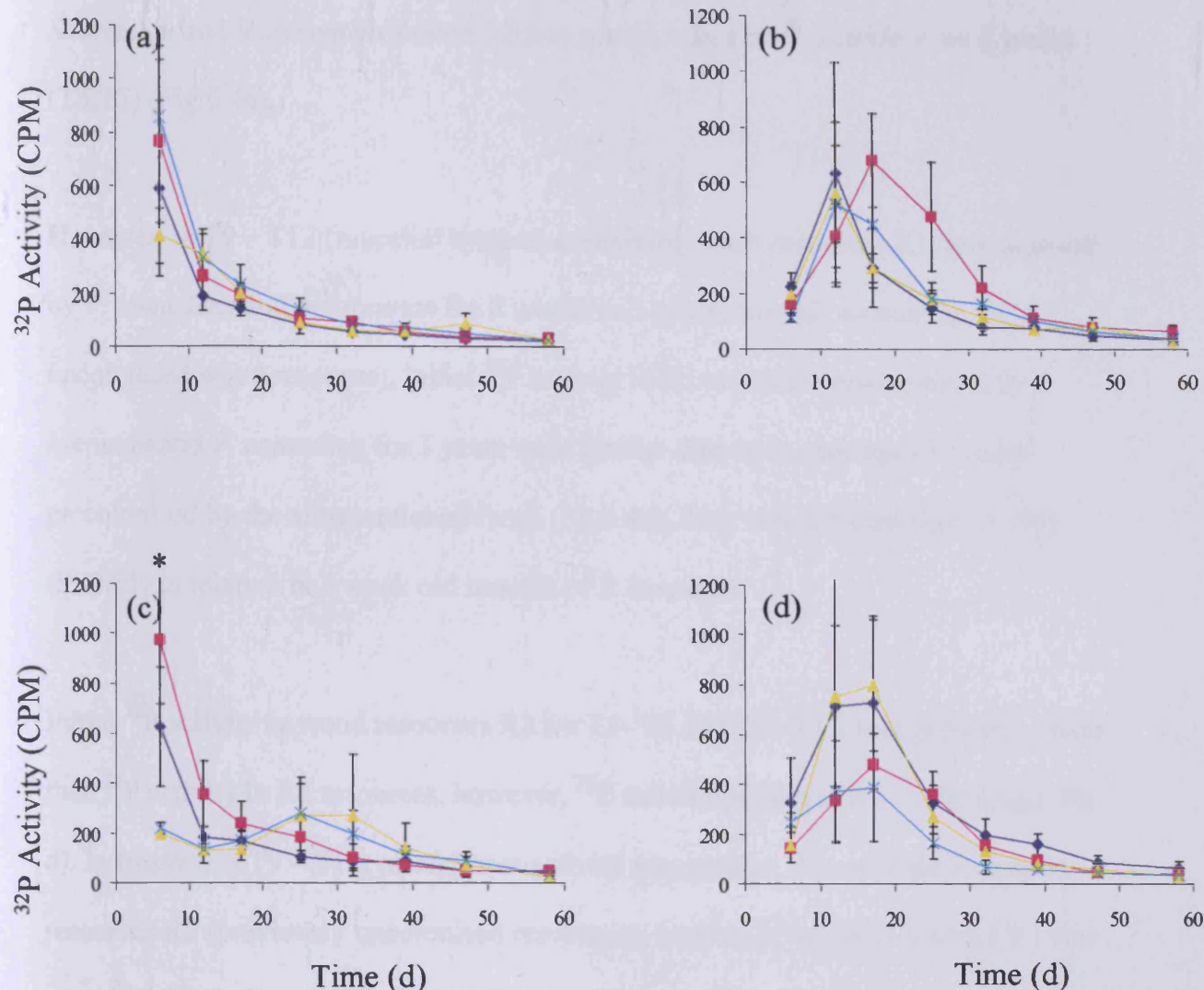


Figure 5.4 The time and course of allocation of soil-derived ^{32}P by *P. velutina* to wood resources R1 (a,c) and R2 (b,d) in mycelial systems containing resources precolonised for 3 years (R1a), 8 weeks (R2b) by *V. comedens* or *S. hirsutum* (T5-T8) and in mycelial systems containing resources colonised for 8 weeks or 3 years by *V. comedens* and *S. hirsutum* (R1c) and a previously uncolonised wood resource (R2d) (T9-T12). Radioactivity was monitored non-destructively * denotes significant difference ($P \leq 0.05$) between means, following One-way ANOVA and Tukey's pairwise comparisons. Symbols for treatments are as follows; T5, T9 (◆), T6, T10 (■), T7, T11 (▲) and T8, T12 (×). Refer to key below:

Symbol	Treatment (T)	Resource 1 (R1)	Resource 2 (R2)
◆	5	<i>S. hirsutum</i>	<i>V. comedens</i>
■	6	<i>S. hirsutum</i>	<i>S. hirsutum</i>
▲	7	<i>V. comedens</i>	<i>V. comedens</i>
×	8	<i>V. comedens</i>	<i>S. hirsutum</i>
◆	9	<i>V. comedens</i>	Uncolonised Wood Resource
■	10	<i>S. hirsutum</i>	Uncolonised Wood Resource
▲	11	<i>V. comedens</i>	Uncolonised Wood Resource
×	12	<i>S. hirsutum</i>	Uncolonised Wood Resource

S.hirsutum and *V.comedens* where R2 was precolonised by *V.comedens* for 8 weeks (T5,T7) (Fig.5.4a).

However, in T9 – T12 (mycelial systems containing wood resources R1; precolonised by *V. comedens* and *S. hirsutum* for 8 weeks or 3 years, and R2; containing an uncolonised wood resource), initial ^{32}P activity in R1 resources precolonised by *S. hirsutum* and *V. comedens* for 3 years were greater than resources aged 8 weeks precolonised by the aforementioned fungi (Fig5.4c). This was detected significantly ($P<0.05$) in relation to 8 week old inocula of *S. hirsutum*.

Initial ^{32}P activity in wood resources R2 for T5- T8 and T9 –T12, was generally lower than ^{32}P activity in R1 resources, however, ^{32}P activity peaked in R2 at 12d (Fig.5.4b, d). In treatments T9 – T11, phosphorus activity was greater, but not significantly in resources R2 (previously uncolonised resources), relating to treatments where R1 was precolonised by *V. comedens* for 8 weeks and 3 years (T9 and T11) than resources precolonised by *S. hirsutum* for both ages (Fig.5.4 d).

5.3.3 Phosphorus allocation at harvest

No significant differences between ^{32}P activity at harvest were detected ($P<0.05$) in mycelial systems of *P. velutina* containing a precolonised wood resource by either *Vuilleminia comedens* or *Stereum hirsutum*, of ages 8 weeks or 3 years (Fig.5.5). However it did appear that ^{32}P activity was greater, but not significantly ($P>0.05$) in resources precolonised by *V. comedens* for both ages, than in resources precolonised by *S. hirsutum*.

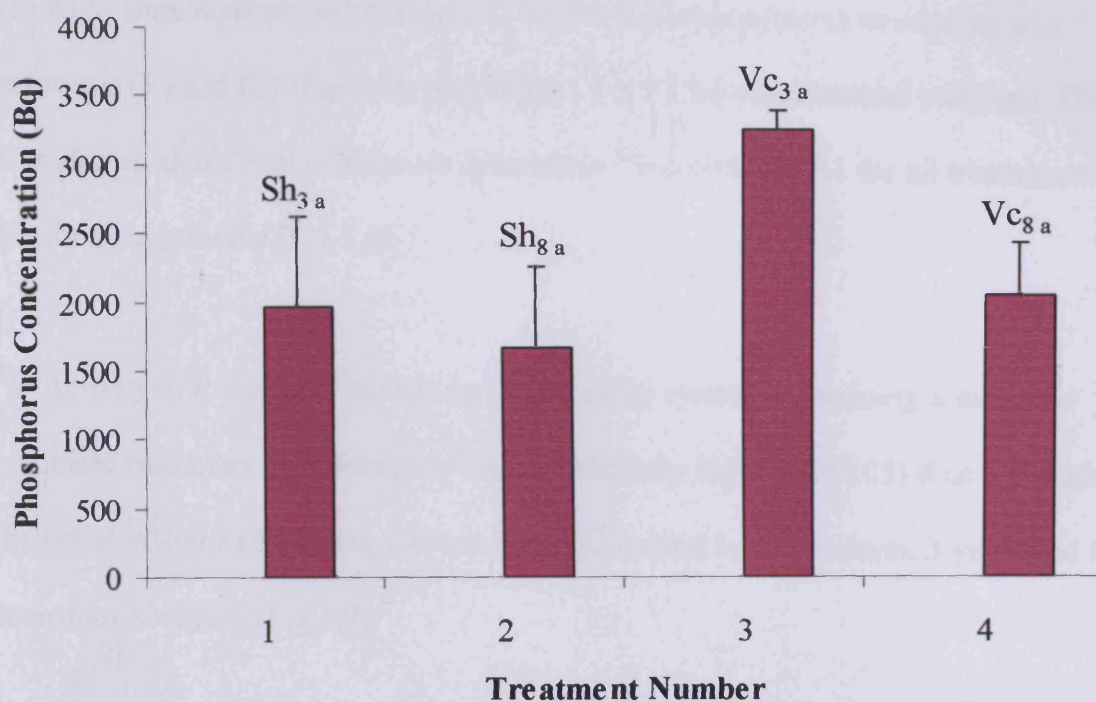


Figure 5.5 ^{32}P concentration (Bq) in wood resources (R_1) precolonised by *Stereum hirsutum* (Sh) or *Vuilleminia comedens* (Vc) for 3 years or 8 weeks in treatment 1-4 (see Table 5.1 p 97). Bars with the same letter (a) are not significantly different ($P < 0.05$).

Due to large variation in ^{32}P activity in wood resources, no significant differences ($P < 0.05$) were detected in treatments 5 – 12 in mycelial systems containing two resources (R_1 and R_2) (Fig.5.6), (see Table 5.1 p 97 for experimental pairings). There was also no significant differences detected in ^{32}P activity in R_1 for all treatments and R_2 for all treatments (Fig.5.6).

^{32}P Activity in *P. velutina* inocula in T2 (mycelial systems containing 8 week old colonised resources by *S. hirsutum*) was significantly higher ($P < 0.05$) than T5 inocula (mycelial systems containing two resources colonised by *S. hirsutum*; 3 years and *V. comedens* 8 weeks) (Fig.5.7).

No significant differences in ^{32}P activity in *P. velutina* inocula were detected between T1-T4, T5-T8 and between T9-T12 ($P > 0.05$). However, significant differences were detected in ^{32}P activity between pooled treatments; T1 to T4, T5 to T8 and T9 to T12, where activity was significantly greater in pooled T1 to T4 than T5 to T8 ($P < 0.05$) (Fig.5.7).

No significant difference was detected in the total ^{32}P uptake at harvest in all wood components between all treatments ($P < 0.05$) (appendix III a). Precolonised resources of *S. hirsutum* aged 3 years in T5, (second resource was precolonised by *V. comedens* 8 weeks) was significantly ($P < 0.05$) lower than precolonised resources of *S. hirsutum* aged 3 years in T10 (second resource uncolonised). No significant differences were detected between treatments for *P. velutina* inocula and second resources ($P > 0.05$) (appendix III a).

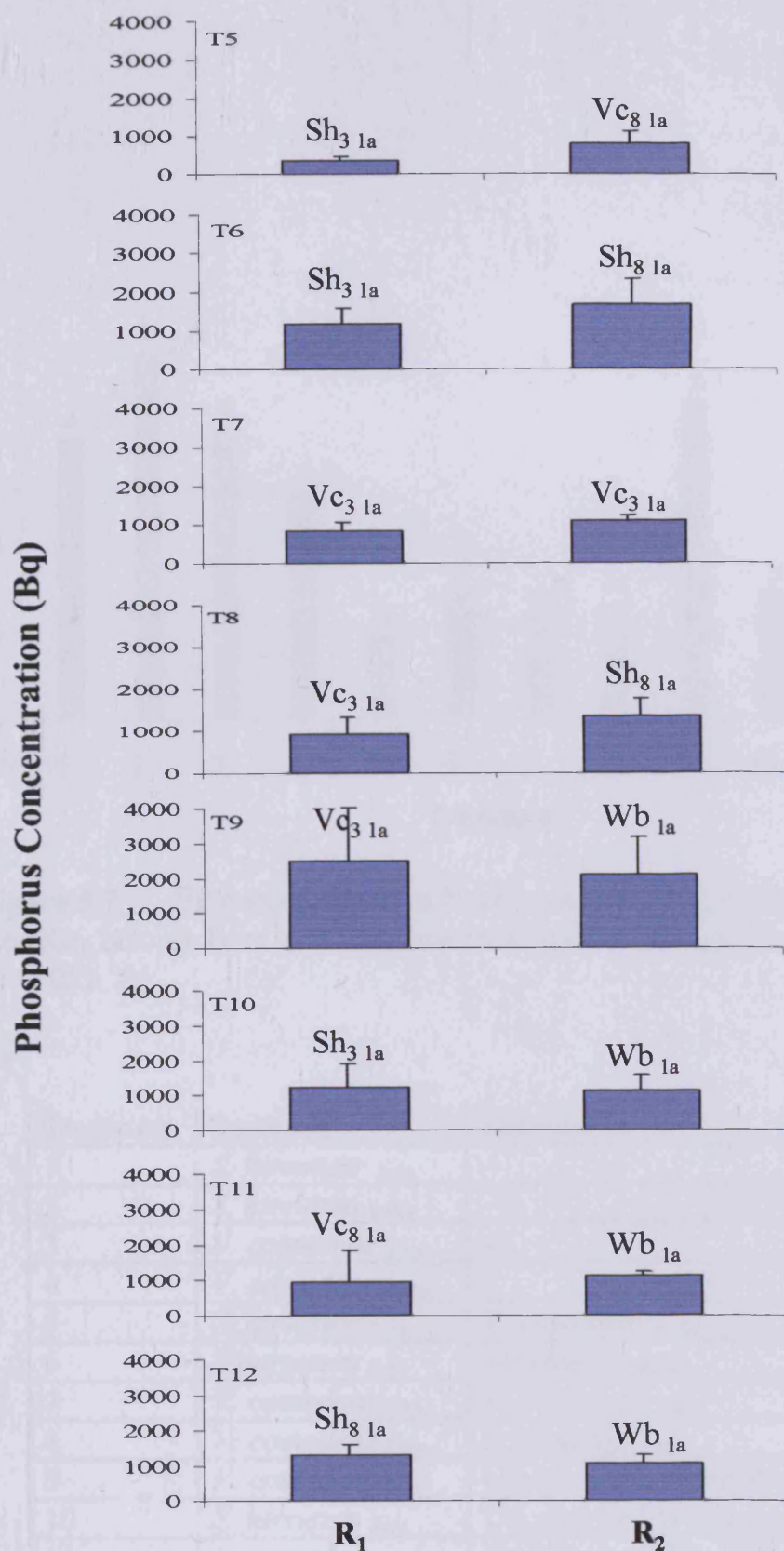


Figure 5.6 ^{32}P concentration in precolonised beech wood resources (R_1 and R_2) for treatments 5 - 12 (see Table 5.1 p 97). R_1 Bars and R_2 bars for all treatments with the same number (1) and treatment with the same letter (a) within each treatment between R_1 and R_2 are not significantly different ($P < 0.05$).

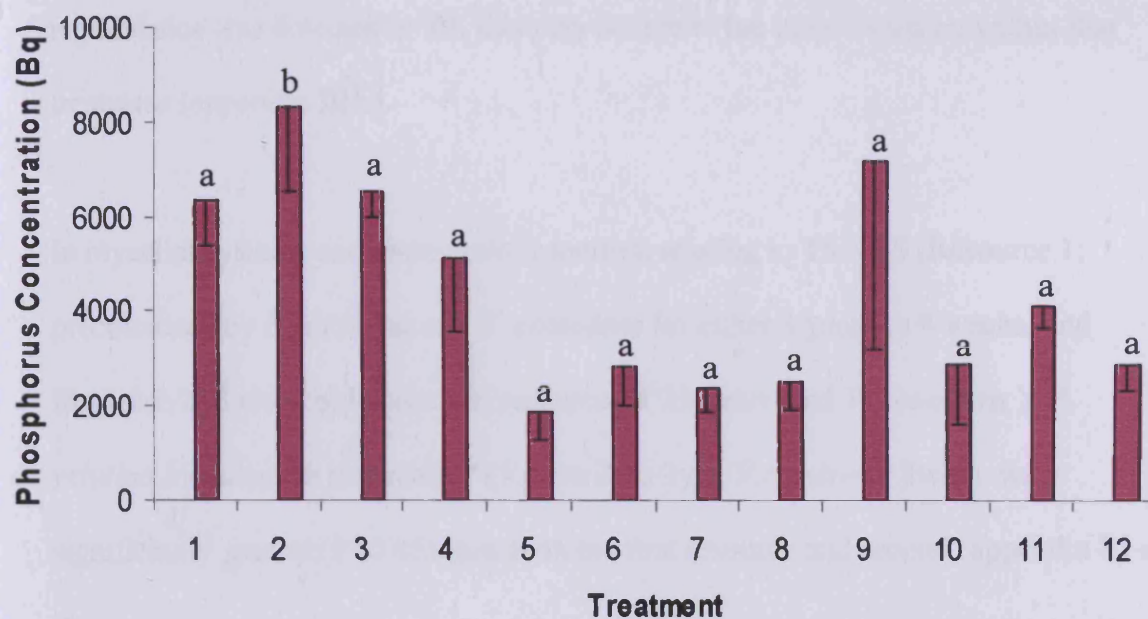


Figure 5.7 ^{32}P concentration in *P. velutina* inoculum in treatments 1-12 (see Key below). Bars with the same letter (a) are not significantly different ($P < 0.05$).

Treatment	Resource 1	Resource 2
1	<i>S. hirsutum</i> 3yrs	-
2	<i>S. hirsutum</i> 8wks	-
3	<i>V. comedens</i> 3yrs	-
4	<i>V. comedens</i> 8wks	-
5	<i>S. hirsutum</i> 3yrs	<i>V. comedens</i> 8wks
6	<i>S. hirsutum</i> 3yrs	<i>S. hirsutum</i> 8wks
7	<i>V. comedens</i> 3yrs	<i>V. comedens</i> 8wks
8	<i>V. comedens</i> 3yrs	<i>S. hirsutum</i> 8wks
9	<i>V. comedens</i> 3yrs	Uncolonised Wood Resource
10	<i>S. hirsutum</i> 3yrs	Uncolonised Wood Resource
11	<i>V. comedens</i> 8wks	Uncolonised Wood Resource
12	<i>S. hirsutum</i> 8wks	Uncolonised Wood Resource

Mycelial systems containing a single precolonised resource, colonised by either *S. hirsutum* and *V. comedens* for 3 years or 8 weeks (T1 – T4), ^{32}P activity in *P. velutina* inocula for T1 – T3, were significantly higher than the precolonised resource. No significance was detected in T4, this may be due to the large variation within that treatment (appendix III a).

In mycelial systems containing two resources, relating to T5 – T8 (Resource 1; precolonised by *S. hirsutum* and *V. comedens* for either 3 years or 8 weeks, and Resource 2; 8 week old colonised resources *S. hirsutum* and *V. comedens*), *P. velutina* inoculum in treatment 7 (*V.comedens* 3yrs, *V.comedens* 8wks) was significantly greater ($P<0.05$) than both the first resource and second (appendix III a).

^{32}P activity in *P. velutina* inocula for treatment 10 (where R1; *S. hirsutum*, 3 years and R2; uncolonised resource) were significantly greater than activity in the first resource and second ($P<0.05$) (appendix III a).

No significant differences were detected in the percentage of the total uptake to previously uncolonised wood resources, *S. hirsutum*, *V. comedens* of 3 years old and 8 weeks old in relation to the other bait contained in the mycelial systems (appendix III b).

5.3.4 Wood decay rates at harvest of *P. velutina* and resources R_1 and R_2

No significant differences were detected for all treatments in wood decay rates of *P. velutina* inoculum ($P>0.05$) (Table 5.3).

Wood decay rates for resources R₁ in T1 (mycelial systems containing resources precolonised by *S. hirsutum* for 3 years), were significantly lower ($P < 0.001$) than treatments T7 (mycelial systems containing resources precolonised by *V. comedens* for 3 years and *V. comedens* for 8 weeks) and T10 (mycelial systems containing resources precolonised by *S. hirsutum* for 3 years and a previously uncolonised wood resource) (Table 5.3).

Wood resources R₂ in T6 (mycelial systems containing resources precolonised by *S. hirsutum* for 3 years and *S. hirsutum* for 8 weeks) was significantly higher ($P < 0.05$) than T9 (mycelial systems containing resources precolonised by *V. comedens* for 3 years and a previously uncolonised wood resource) (Table 5.3)

5.3.5 Outcome of mycelial interactions in precolonised wood following an encounter by *P. velutina*

Having determined the degree of mycelial replacement in wood resources at the end of the experiment (see section 2.10), generally mycelial in all treatments; T3, 4, 5, 7, 8, 9 & 11, containing resources precolonised by *V. comedens* were fully replaced by *P. velutina* (Table 5.4 & 5.5). Treatments containing 3year old precolonised resources by *S. hirsutum* (T1), two out of the five resources were fully replaced by *P. velutina*, two were partially replaced and one resource no replacement occurred (Table 5.4). Similarly in T2, two resources were fully replaced by *P. velutina* and three were partially replaced.

Treatment	Treatment Pairings			Relative Wood Decay Rate (mg g ⁻¹ d ⁻¹)		
	R1	Pv	R2	R1	Pv	R2
1	Sh 3yrs	Pv		3.934±0.92 ac***	4.928±0.50	
2	Sh 8wks	Pv		5.786±0.17 a	4.995±0.63	
3	Vc 3yrs	Pv		8.030±0.83 a	4.913±0.21	
4	Vc 8wks	Pv		6.733±0.09 a	5.276±0.24	
5	Sh 3yrs	Pv	Vc 8wks	4.274±1.03 a	5.044±0.35	6.482±0.30 a
6	Sh 3yrs	Pv	Sh 8wks	4.274±1.04 a	4.995±0.63	7.098±1.56 ab*
7	Vc 3yrs	Pv	Vc 8wks	8.720±0.50 ab	5.812±0.48	7.020±0.18 a
8	Vc 3yrs	Pv	Sh 8wks	8.107±0.65 a	5.363±0.39	6.338±0.18 a
9	Vc 3yrs	Pv	Wb	9.027±1.01 a	4.768±0.19	4.770±0.33 ac
10	Sh 3yrs	Pv	Wb	6.206±0.48 ab	4.536±0.38	3.983±0.63 a
11	Vc 8wks	Pv	Wb	4.307±0.35 a	5.566±0.23	5.327±0.27 a
12	Sh 8wks	Pv	Wb	5.702± 0.58 a	5.710±0.27	4.120±0.38 a

Table 5.3 Data represented are a summary for 5 replicate mycelial systems of *P. velutina* (Pv) where one or two resources (R1 & R2) were added behind the colony growing front. Decay data are represented with the standard error of the mean (n=5). Figures in the same column followed by the same letter are not significantly different (P<0.05 *, P<0.01 **, P<0.001***) following one-way ANOVA and Priori tests.

In treatments containing both resources precolonised by *Stereum hirsutum* but of different ages (T6), the same outcome of interaction was recorded. The mycelia in three resources were fully replaced by *P. velutina* and the remaining two were partially replaced. In T5 (mycelial systems containing *S. hirsutum*; 3 years and *V. comedens*; 8 weeks), two resources previously containing mycelia of *S. hirsutum* were fully replaced and one partially replaced by *P. velutina*. *Stereum hirsutum* persisted to fully colonise the remaining resource, there was no evidence that *P. velutina* was present (Table 5.4 & 5.5). In T10 (mycelial systems containing *S. hirsutum*; 3 years and a previously uncolonised resource), 2 of the resources previously containing *S. hirsutum* were fully replaced by *P. velutina*, one was partially replaced and the remaining two had no evidence of colonisation by *P. velutina* (Table 5.4 & 5.5).

Treatment	Precolonised resource (R ₁)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
1	Sh 3yrs	100	100	75	25	0
2	Sh 8wks	100	100	75	50	50
3	Vc 3yrs	100	100	100	100	100
4	Vc 8wks	100	100	100	100	100
5	Sh 3yrs	100	100	75	0	0
6	Sh 3yrs	100	100	100	75	75
7	Vc 3yrs	100	100	100	100	100
8	Vc 3yrs	100	100	100	100	100
9	Vc 3yrs	100	100	100	100	100
10	Sh 3yrs	100	100	50	0	0
11	Vc 8wks	100	100	100	100	100
12	Sh 8wks	100	100	100	50	50

Table 5.4 Outcome of mycelial interactions between *P.velutina* and precolonised resources (R₁) for treatments 1-12 (Table 5.1) of *Stereum hirsutum* and *Vuilleminia comedens* at ages 8 weeks and 3 years

Treatment	Precolonised / uncolonised resource (R ₂)	Percentage colonisation by <i>P. velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	Vc 8wks	100	100	100	100	100
6	Sh 8wks	100	100	100	50	25
7	Vc 8wks	100	100	100	100	100
8	Sh 8wks	100	100	100	75	75
9	Wb	100	100	100	100	100
10	Wb	100	100	100	100	100
11	Wb	100	100	100	100	100
12	Wb	100	100	100	100	100

Table 5.5 Outcome of mycelial interactions between *P. velutina* and precolonised resources of *Stereum hirsutum* and *Vuilleminia comedens* at ages 8 weeks and colonisation by *P. velutina* of previously uncolonised wood resources, (R₂) for treatments 5-12 (Table 5.1).

5.4 DISCUSSION

We know from qualitative and quantitative studies that re-deployment of mycelial biomass and phosphorus translocation can be affected by addition of wood resources uncolonised or pre-colonised by other wood decay fungi (Chapter 3 and 4). Wood resources already colonised arriving on an established system of *P. velutina* can have major effects on ^{32}P uptake and translocation to and from the occupier of the resource encountered by *P. velutina* (Wells, Hughes & Boddy, 1990, chapter 4)

In earlier studies there is evidence that decay state and age of inoculum play an important factor in determining the nutrient pathways of mycelial systems. When encountering newly fresh previously uncolonised resources, and baits of different quality such as leaf litter, phosphorus translocation in mycelial cord systems of *P. velutina* and *P. impudicus* is dependent on the inoculum species, ie, decay state/quality of the inoculum and also resource quality encountered (Wells & Boddy, 1990; Wells, Hughes & Boddy, 1990). Earlier qualitative studies have also demonstrated that *P. velutina* exhibits preferential colonisation of pre-colonised resources of different decay state and age (Boddy & Abdalla, 1998). However, studies on nutrient translocation to such resources i.e. in relation to the time of colonisation by other wood decay fungi are limited.

This study has demonstrated that ^{32}P translocation to resources pre-colonised by other wood decay fungi of two inoculum ages, 8 weeks and 3 years, and the arrival of one or more woody resources of different quality (i.e. decay state and age) on established mycelial systems, significantly affects the amount of phosphorus translocation to

these resources. Phosphorus translocation is significantly affected by the presence or absence of other wood decay fungi where *P. velutina* will preferentially invade the least combative fungus.

Initially greater phosphorus translocation to woody resources precolonised by *V. comedens* for three years, when compared to other treatments, was detected irrespective of whether mycelial systems contained one or two wood resources. This was detected also in mycelial systems containing two resources where ^{32}P was greater initially in wood resources precolonised by *S. hirsutum* for three years. This study suggests that *P. velutina* favoured phosphorus translocation to the much older precolonised wood resources when compared to 8 week old inocula. Therefore there is evidence that time of colonisation does affect phosphorus translocation in mycelial systems of *P. velutina*.

Generally it was noted that phosphorus activity in wood resources colonised for three years by *V. comedens* and *S. hirsutum* resources declined after 12 d and an influx of phosphorus occurred after 12 d particularly in second resources, either un-colonised or pre-colonised for 8 weeks (T5-8). A possible explanation for the reallocation of nutrients to the younger resources may be due to lessor combative actions exhibited in these resources. Initially the demand for phosphorus was directed towards the older inoculum rather than the resource only precolonised for 8 weeks, as a more combative response was required by *P. velutina* in order to invade the wood resource occupied by the other wood decaying fungus.

In systems containing 3 year old inocula of *V. comedens* and *S. hirsutum*, where the second resource was a previously un-colonised resource, greater translocation initially

to the older pre-colonised resources for both *V. comedens* and *S. hirsutum* were observed. In particular, significantly greater activity was detected in resources containing 3 year old *S. hirsutum* when compared to 8 week old *S. hirsutum*. This initial high level of ^{32}P translocation to 3 year old pre-colonised wood resource may be due to the combative response exhibited by *P. velutina*, where a greater need for nutrients are required in order to invade and gain occupancy of the new resources encountered. This trend was exhibited in treatments containing 3 year *V. comedens* and *S. hirsutum* where the second resource contained the precoloniser *S. hirsutum*, however, this was not detected significantly.

At the end of the experiment, in mycelial systems containing only one resource, phosphorus activity was greater (but not significantly) in resources pre-colonised by both ages (8 weeks & 3 years) by *V. comedens*, and appeared to be greater at harvest in resources aged 3 years in respect to *V. comedens* and *S. hirsutum*. As hypothesised, when given a choice of wood resource *P. velutina* will preferentially invade the resource that offers the least combative response. In order of highest phosphorus activity, these were as follows *V. comedens* (3yrs) > *V. comedens* (8wks) > *S. hirsutum* (3yrs) > *S. hirsutum* (8 wks).

In this study, in relation to outcome of mycelial interactions in wood resources, there was evidence of preferential colonisation of resources previously occupied by *V. comedens* irrespective of inoculum age, however, older inoculum precolonised by *S. hirsutum* were less colonised when compared to its younger resources of 8 weeks. A preference for colonisation of younger resources in terms of *S. hirsutum* has also been reported by Boddy and Abdalla (1990), where younger inocula of 6 weeks

precolonised by *S. hirsutum* was preferentially colonised in respect to two year old wood resources containing the pre-coloniser *S. hirsutum*.

In a study conducted by Hughes & Boddy (1994) it was shown that there is a demand for phosphorus during the early stages of colonisation, indicating also that state of decay can influence phosphorus translocation in mycelial systems. Hughes & Boddy monitored ^{32}P activity in mycelial systems of *P. velutina* extending from 1cm³ inocula containing two previously uncolonised fresh sterile wood blocks, added at 10 d intervals to mycelial systems, where phosphorus activity was initially greater in the second wood bait rather than the first. Interestingly, in this study, greatest ^{32}P activity has been detected in the older resources of *S. hirsutum* when compared to 8 week old precolonised resources of *S. hirsutum*, a possible explanation is that there also a high demand for phosphorus during the stages of combat. This study has also demonstrated that phosphorus is also translocated in preference to favourable, 'safe' resources which contain a lessor combative fungus such as *V. comedens* rather than a resource less easily to invade.

CHAPTER 6

Phosphorus translocation by *Phanerochaete velutina* encountering unit restricted fungi on soil: Effect of inoculum size

6.1 INTRODUCTION

Numerous studies have demonstrated that the abundance of woody material on mycelial cord systems, for example wood resources, litter packs can increase rate of decay of the original inoculum (Wells, Boddy & Donnelly, 1997; Boddy & Abdalla, 1998, Hughes & Boddy, 1996), and the presence or absence of other wood decay fungi in wood resources can influence nutrient movement to and from resources encountered (Chapter 5, Wells & Boddy, 1990; Wells & Boddy; 1995a,b,c).

The affect on ^{32}P allocation and uptake can be influenced by the simultaneous addition of one or more woody resources, and those resources encountered being of different size (Hughes & Boddy 1996). We know from these studies that the affect of addition of wood resources is to cause cessation of mycelial extension rates, and regression of mycelium within the systems (Wells, Harris & Boddy, 1998).

In a study in relation of the effect of inoculum size (Hughes & Boddy 1994), resources of different sizes (0.5, 1, 2, and 4 cm³) and quality (resources precolonised by *P. velutina* 1 or 2 cm³; same genotype as central inoculum) were placed on foraging mycelial systems of *P. velutina* extending from 1cm³ inocula. Hughes & Boddy

(1994), reported that total mycelial uptake of phosphorus of the whole system is dependent on the size of the bait to which ^{32}P was supplied. Small baits had less demand for phosphorus when compared to larger baits encountered. Preferential phosphorus allocation was evident if the bait was larger than 2 cm^3 and was previously un-colonised.

Mycelial systems of *P. velutina* extending from inoculum of different volumes was investigated in relation to Phosphorus uptake by *P. velutina* and translocation to and between wood resources of 4 cm^3 precolonised by the wood decay fungi of different combative abilities. This study aimed to answer the following questions: (1) Does the ratio of inoculum / new resource encountered influence the amount of ^{32}P translocated within mycelial systems? 2) Does the quality of the resource affect the outcome of interactions in relation to inoculum size of *P. velutina*?

6.2 MATERIALS AND METHODS

6.2.1 Fungal isolates

Phanerochaete velutina, *Stereum hirsutum*, *Coriolus versicolor* and *Vuilleminia comedens* were originally isolated from decaying beech wood from the Forest of Dean, UK, (National Grid Reference SO 611145). Laboratory stocks were routinely maintained and routinely subcultured as described in section 2.1- 2.2

6.2.2 Preparation of Inocula and precolonised wood resources of different inoculum size and resource quality.

Beech wood blocks $10 \times 10 \times 10 \text{ mm}^3$ and $20 \times 20 \times 10 \text{ mm}^3$ were cut, stored and autoclaved as described in section 2.5. Batches of 25 twice autoclaved wood blocks (size $20 \times 20 \times 10 \text{ mm}^3$) at 24hr intervals were transferred aseptically into 14cm diameter Petri dishes containing two week old cultures, grown on MA of either *S. hirsutum*, *C. versicolor* and *V. comedens*. Wood blocks of both sizes ($10 \times 10 \times 10 \text{ mm}^3$ and $20 \times 20 \times 10 \text{ mm}^3$) were transferred to 14 cm dishes of MA colonised for two weeks by *P. velutina*, 4 wood blocks of size $20 \times 20 \times 10 \text{ mm}^3$ were positioned together ($40 \times 40 \times 10 \text{ mm}^3$; 16 cm^3) on MEA dishes colonised by *P. velutina*. The dishes were then incubated at 20°C for 8 weeks to ensure colonisation of the wood.

6.2.3 Preparation of mycelial systems

Petri dish bases, 90mm diameter, were glued centrally inside the 24 cm^2 Perspex trays. These acted as radioisotope compartments. Non-sterile soil was collected and prepared as described in section 2.8.1-2.8.2 (Figure 6.1).

Eight week old *Phanerochaete velutina* inocula were removed from the 14cm dishes, and were prepared for the addition onto compressed soil as described in section 2.8.3. *P. velutina* inocula, of three inoculum sizes (Table 6.1) were placed firmly into a central position contained within 90mm radioisotope compartment. 1 cm^3 inocula were added 3 d and 8 d prior to the inoculation of soil trays with 4 and 16 cm^3 wood blocks. This was to ensure mycelial colonies were of similar diameter when pre-colonised resources were added (section 6.2.5).

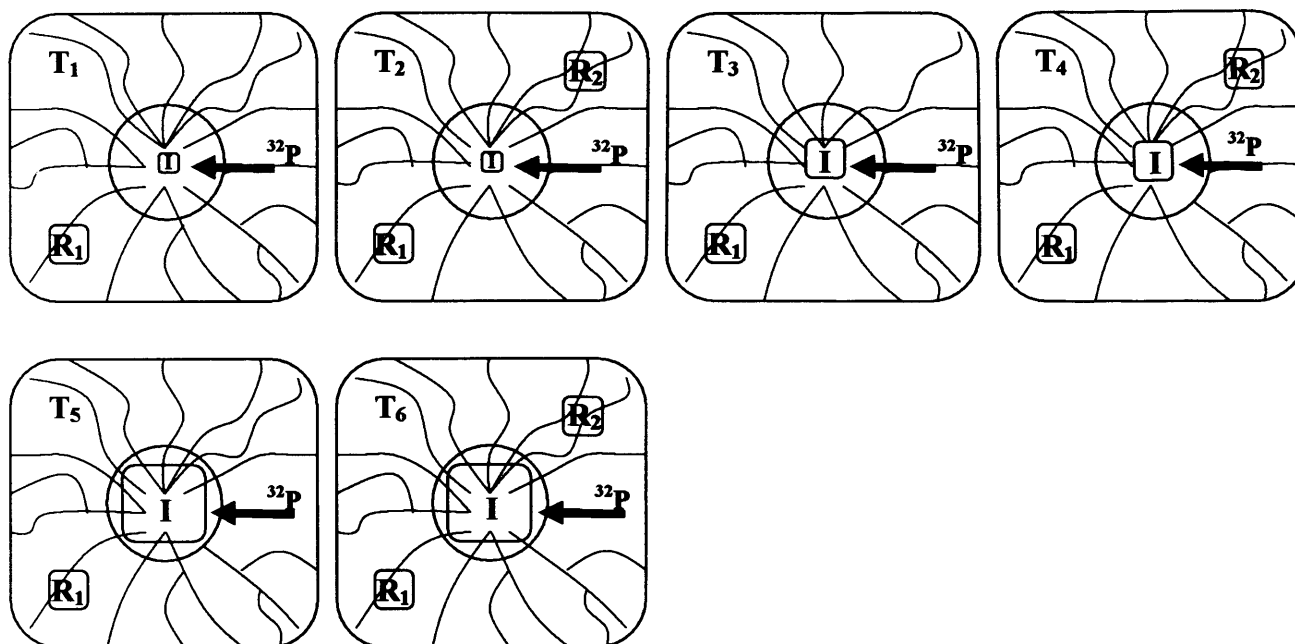


Figure 6.1 Diagram representing the experimental set-up of soil systems inoculated with wood resources colonised by *P. velutina* (I). Six designs were employed (a, b, c, d, e & f) where wood resources precolonised (R1) and/ or precolonised / uncolonised (R2) were added to the mycelial systems of *P. velutina* of inoculum size 1cm³ (a, b) 4cm³ (c, d) and 16cm³ (e, f). ^{32}P labelled solution was added to the soil around *P. velutina* inocula. To the remaining soil, unlabelled phosphorus solution was added.

When mycelial systems reached the edge of the soil dish (1 cm³, 36 d; 4 cm³, 33 d and 16 cm³, 28 d), uncolonised, or precolonised resources of constant size (4cm³), were placed onto the mycelial system, behind the mycelial margin (see Table 6.2 for experimental pairings). Sub-samples of inocula, uncolonised wood and precolonised resources were taken in order to determine Relative Density (g cm⁻³). Inoculated trays were incubated at 20°C as described in section 2.4.

Inoculum Volume (cm ³)	Ratio of inoculum to resource
1	1 : 4
4	1 : 1
16	4 : 1

Table 6.1 Ratio of inoculum to precolonised / uncolonised resources investigated for *Phanerochaete velutina*.

Treatment	Resource 1	Inocula	Resource 2
1	Sh	Pv	
2	Vc	Pv	
3	Cv	Pv	
4	Wb	Pv	
5	Sh	Pv	Vc
6	Cv	Pv	Vc
7	Cv	Pv	Sh
8	Sh	Pv	Wb
9	Vc	Pv	Wb
10	Cv	Pv	Wb

Table 6.2 Experimental design for the effect of inoculum size on phosphorus translocation and the outcome of interaction where inocula of *P. velutina* of three sizes (Pv) encountered combinations of *Stereum hirsutum* (Sh), *Vuilleminia comedens* (Vc), *Coriolus versicolor* (Cv) and uncolonised wood blocks (Wb). Five replicas for each treatment.

6.2.4 Addition of ^{32}P to mycelial systems

To 38, 35 and 28 d old mycelial systems of *P. velutina* (1, 4 and 16 cm³ respectively), two days after the addition of uncolonised / precolonised resources, ^{32}P solution was added around the central inoculum which covered 1/8th of the soil for each mycelial system (Fig 6.1 – 6.2).

A stock solution of 20mM $\text{NH}_4\text{K}_2\text{PO}_4$ (AnalaR) was made to a volume of 1 litre. 100 ml of stock solution contained 159 M.Bq ^{32}P orthophosphate (Amersham, carrier-free in dilute HCL). 8 x 50 μl aliquots of the labeled solution was supplied uniformly to the soil within the 5cm diameter radioisotope compartment; 0.64 M.Bq ^{32}P orthophosphate / tray. To the remainder of the soil tray, 56 x 50 μl of unlabeled 20mM $\text{NH}_4\text{K}_2\text{PO}_4$ (AnalaR) was added uniformly through evenly spaced holes drilled through a Perspex 24cm² bioassay lid.

Non-destructive determination of phosphorus activity in wood resources is described in section 2.8.6. Phosphorus activity was monitored for a period of 59 days, after which, all wood components were harvested from the mycelial systems and ^{32}P was determined destructively as described in section 2.8.8.

6.2.5 Phosphorus translocation to precolonised wood resources of different quality: The effect of inoculum size

At 36, 33 and 28d for mycelial systems inoculated with 1, 4 and 16cm³ *P. velutina* inocula respectively, a single wood resource (Figure 6.1; T1, T3, T5) or two wood resources (Fig. 6.1; T2, T4, T6) uncolonised or 8 week old precolonised resources of

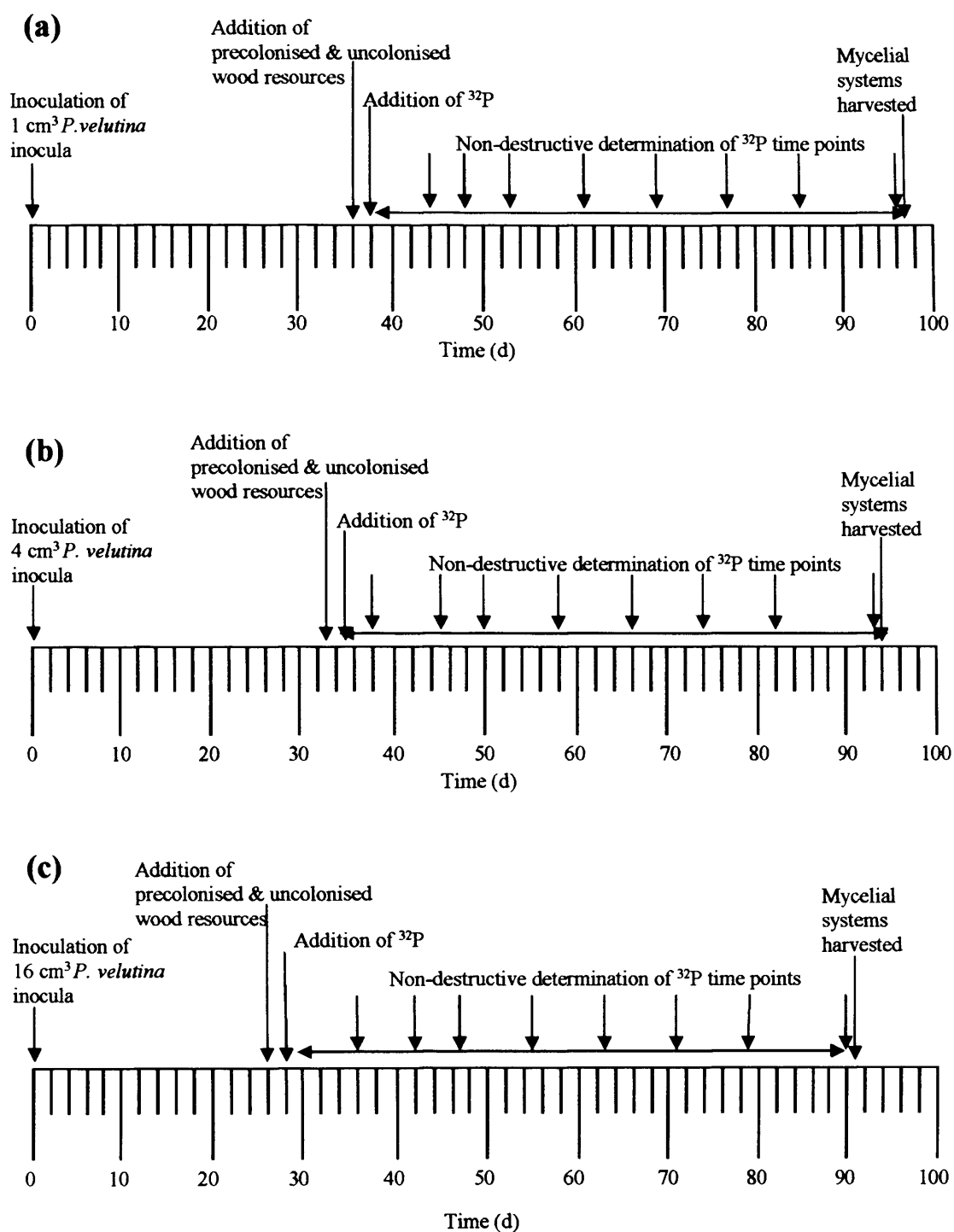


Figure 6.2 Time scale of the addition of ³²P (P) to mycelial systems inoculated with 1 cm³ (a), 4 cm³ (b) or 16 cm³ (c) *P. velutina* colonised wood resources.

Stereum hirsutum, *Coriolus versicolor* or *Vuilleminia comedens* were positioned directly onto the colony at a diagonal from *P. velutina* central inoculum (1, 4 and 16 cm³) 2 cm away from the edge of the tray. In control systems, a fresh previously uncolonised wood resource was placed onto the mycelial system. Five replicates were made of each treatment. The trays were kept in polythene bags to minimise moisture loss and were incubated in the dark at 20°C.

6.2.6 Decay rate and colonisation of beech wood resources

Before the start of the experiment the initial relative density (RD g cm⁻³) of inocula, uncolonised and precolonised resource resources was obtained. Mycelial systems were harvested at 98, 95 and 90 d in relation to systems extending from 1, 4 and 16 cm³ and final RD was determined in order to estimate wood decay rates (g mg³d⁻¹) (Table 6.3).

Wood chips from a sub-sample of pre-colonised wood resources and previously uncolonised resources were plated aseptically onto 2% Malt Extract Agar as described in section 2.10 to determine outcome of mycelial interactions in wood.

Wood Resource un-colonised or colonised by;	Relative Density at the start of experiment (g cm ⁻³)
<i>P. velutina</i> (1cm ³)	0.547
<i>P. velutina</i> (4cm ³)	0.572
<i>P. velutina</i> (16cm ³)	0.544
<i>V. comedens</i> (4cm ³)	0.569
<i>S. hirsutum</i> (4cm ³)	0.589
<i>C. versicolor</i> (4cm ³)	0.564
Uncolonised	0.601

Table 6.3 Relative densities (g cm⁻³) of wood resources, prior inoculation of soil trays of *Phanerochaete velutina* inocula of 1, 4 and 16 cm³, pre-colonised resources and uncolonised wood blocks

6.2.7 Statistical analyses

One-way ANOVA and where appropriate *posteriori* tests were used to compare treatment means, following Levene's test for equality of variances. Kruskal-Wallis tests were employed to compare treatment medians with unequal variances. Statistical analyses were performed using MINTAB for windows version 13.

6.3 RESULTS

6.3.1 Phosphorus translocation in mycelial systems extending from different sized inocula containing one new resource

In systems of *P. velutina* growing from 1cm³ inoculum, ³²P activity, at 12 d after the addition of ³²P to *P. velutina* inoculum, in resources pre-colonised by *C. versicolor* was significantly greater (P≤0.01) than the three other treatments where pre-colonised

resources of *V.comedens*, *S. hirsutum* or a previously un-colonised fresh wood resource were added. This trend persisted until 25 d, however no significant difference between activity levels in resources of *C. versicolor* and *S. hirsutum* were detected at 17 and 25 d ($P>0.05$). (Fig.6.3).

In mycelial systems extending from 4 cm³ inocula, ³²P activity in resources pre-colonised by *C. versicolor* was significantly greater than in resources pre-colonised by *S. hirsutum* ($P<0.05$). Also in systems of *P. velutina* growing from 16cm³ wood resources, ³²P activity in resources previously un-colonised was significantly greater than in resources pre-colonised by *S. hirsutum* ($P<0.05$).

Over time, overall activity in resources pre-colonised by *S.hirsutum* was usually lower than in resources pre-colonised by *C. versicolor* and *V. comedens*.

6.3.2 Phosphorus translocation in mycelial systems extending from different sized inocula containing two new resources

Generally, significant differences in ³²P activity were detected in the second resources in mycelial systems of *P. velutina* growing from 1cm³ ($P\leq 0.05$) and 16 cm³ inoculum ($P\leq 0.05$, $P\leq 0.01$) (Fig.6.4b & f). No significant differences were detected in ³²P activity in the first resources (R1) (Fig 6.4 a, c & d) ($P\geq 0.05$).

In treatments where *P. velutina* growing from inoculum size of 1cm³, containing pre-colonised resources of *C. versicolor* (R1) and *V. comedens* (R2), activity in the second resources pre-colonised by *V. comedens* 12 d after the addition of the radioisotope were

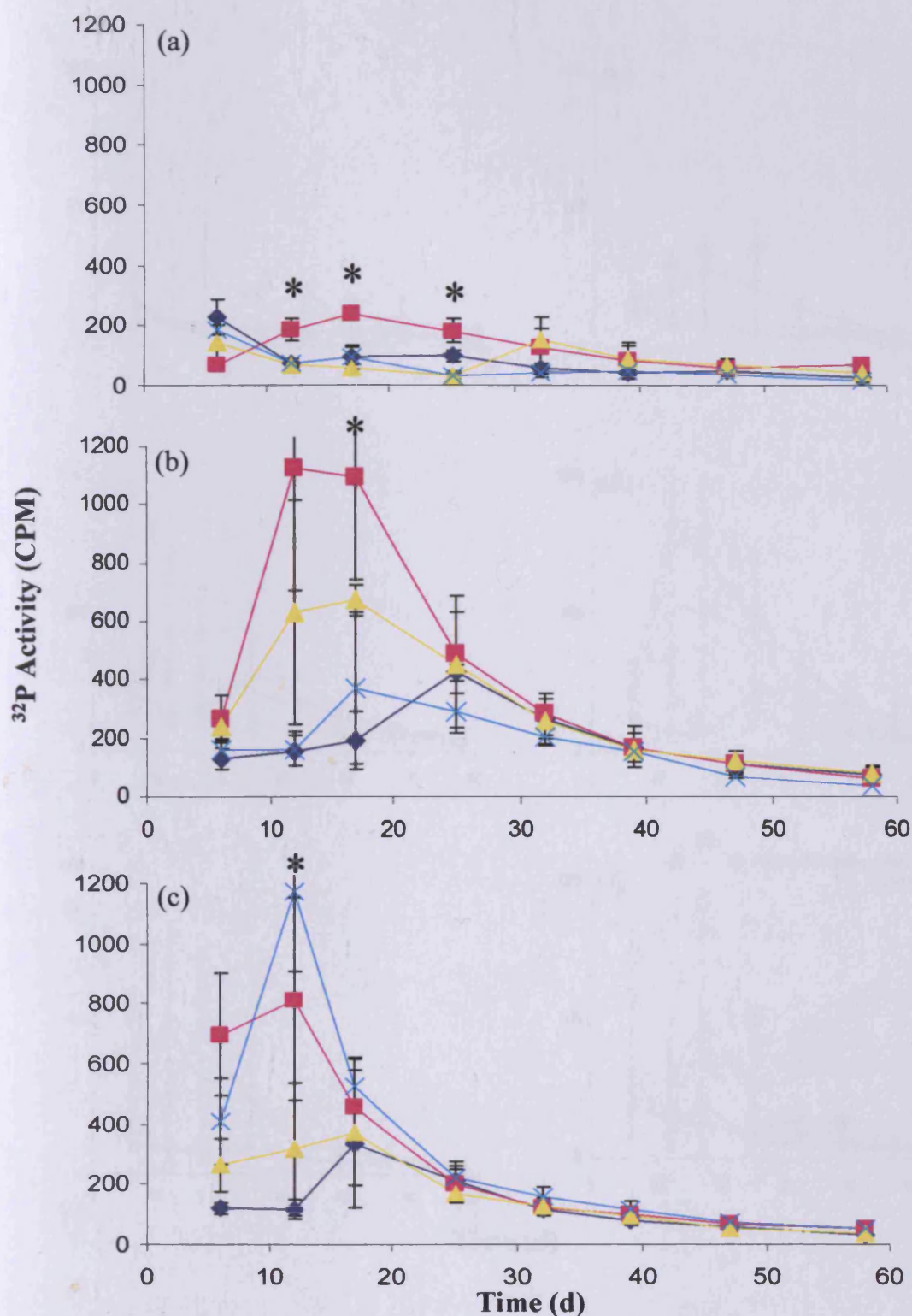


Figure 6.3 The time and course of allocation of soil-derived ^{32}P by *P. velutina* of inoculum size 1 (a), 4 (b) and 16 cm^3 (c) in systems containing a single wood resource, pre-colonised by *Stereum hirsutum* (◆), *Coriolus versicolor* (■) or *Vuilleminia comedens* (▲). Positive control systems were supplied with a previously un-colonised beech wood resource (×). Radioactivity was monitored non-destructively. * denotes significant difference ($P \leq 0.05$) between means, following One-way ANOVA and Tukey's pairwise comparisons.

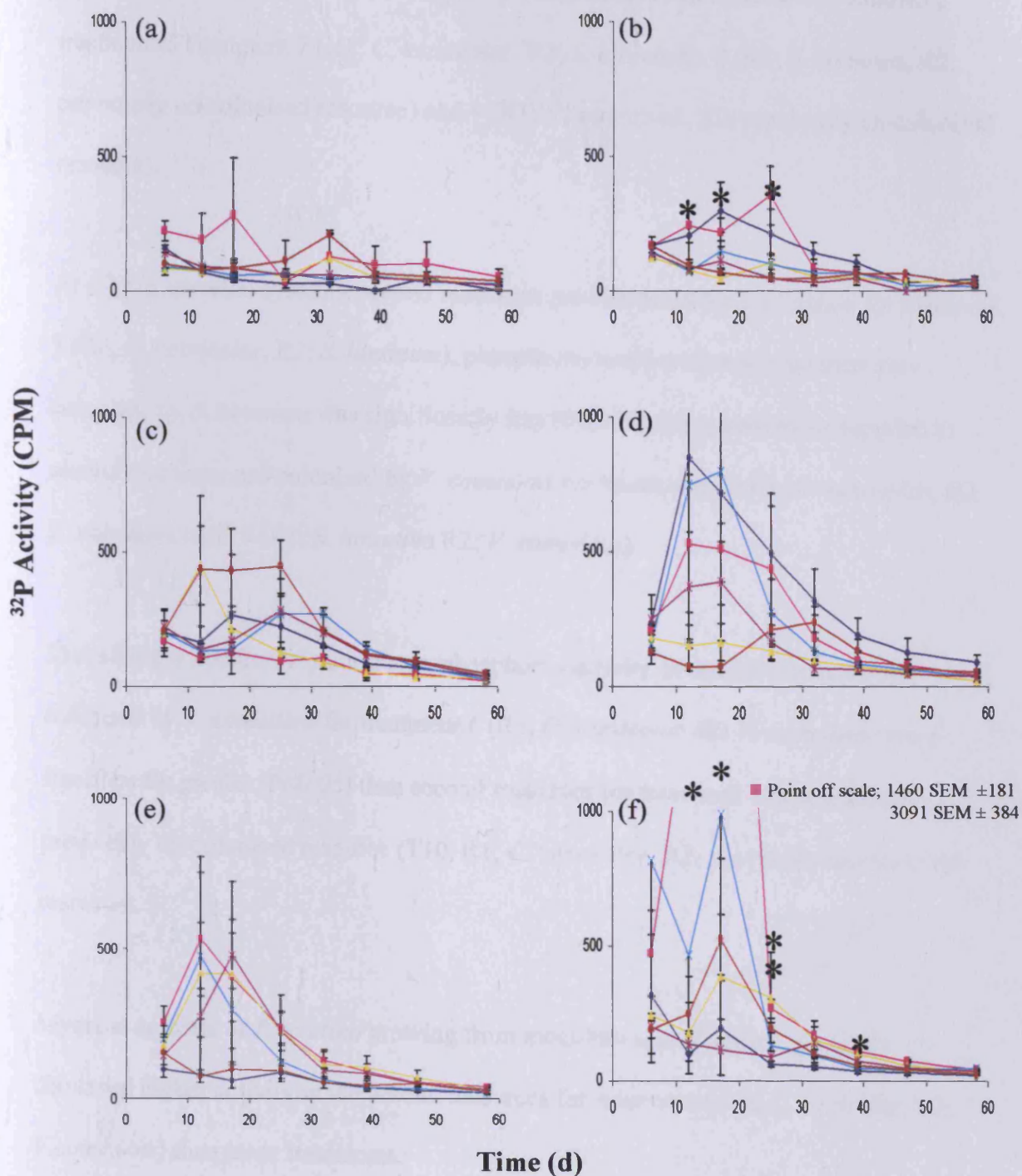


Figure 6.4 The time and course of allocation of soil derived ^{32}P by *P. velutina* of inoculum size 1 (a & b), 4 (c & d) and 16 cm³ (e & f) to wood resources R1 (a, c & e) and R2 (b, d & f) in mycelial systems -containing resources un-colonised and pre-colonised by *Stereum hirsutum*, *Coriolus versicolor* and *Vuilleminia comedens*. Radioactivity was monitored non-destructively. * denotes significant difference ($P \leq 0.05$) between means, following One-way ANOVA and Tukey's pairwise comparisons. Symbols for treatments are as follows; T5 (◆), T6 (■), T7 (▲), T8 (×), T9(*) and T10 (*).

significantly greater ($P \leq 0.05$) than activity in the second resources for the following treatments: Treatment 7 (R1; *C. versicolor*, R2; *S. hirsutum*), 8 (R1; *S. hirsutum*, R2; previously un-colonised resource) and 9 (R1; *C. versicolor*, R2; previously un-colonised resource).

At 17 d in mycelial systems, second resources pre-colonised by *S. hirsutum* for treatment 7 (R1; *C. versicolor*, R2; *S. hirsutum*), phosphorus translocation to resources pre-colonised by *S. hirsutum* was significantly less ($P \leq 0.05$) than phosphorus supplied to second resources pre-colonised by *V. comedens* for treatments 6 (R1; *C. versicolor*, R2; *V. comedens*) and 7 (R1; *S. hirsutum* R2; *V. comedens*).

25 d after the addition of radiotracer, phosphorus activity in second resources pre-colonised by *V. comedens* for treatment 6 (R1; *C. versicolor*, R2 *V. comedens*) were significantly greater ($P \leq 0.05$) than second resources for treatment 10 containing a previously un-colonised resource (T10, R1; *C. versicolor*, R2; previously un-colonised resource).

Mycelial systems of *P. velutina* growing from inoculum size of 16cm³, generally displayed higher activity in the second resources for treatment 6 R1; *C. versicolor*, R2; *V. comedens*) than other treatments.

Phosphorus activity in second resources at 12 d pre-colonised by *V. comedens* (T5 where R1; *S. hirsutum*), and previously un-colonised resource (T 9, where R1; *V. comedens* and T10, where R1; *C. versicolor*) was significantly less than activity detected in second resources pre-colonised by *V. comedens* in treatment 6 where R1

contained the pre-coloniser *C.versicolor* ($P \leq 0.05$). This trend continued at 25d ($P \leq 0.01$) and 39 d ($P \leq 0.05$) where second resources in treatment 6; containing the pre-coloniser *V.comedens*, displayed significantly greater activity than second resources for treatment 5 (R1; *S.hirsutum*, R2; *V.comedens*) and 9 (R1; *V.comedens*, R2; previously un-colonised wood resource).

At 25d, phosphorus activity in second resources for treatments 7 (R1; *C.versicolor*, R2; *S.hirsutum*) and 10 (R1: *C.versicolor*, R2; previously un-colonised wood resource) were significantly greater than activity in second resources pre-colonised by *V. comedens* of treatment 5 (R1; *S. hirsutum*, R2; *V. comedens*).

6.3.3 Phosphorus allocation at harvest in mycelial systems containing one or two pre-colonised wood resource (R1 & R2)

No significant differences were detected between ^{32}P activity in *P. velutina* inoculum for all treatments regardless of inoculum size of 1, 4 or 16 cm³ ($P \geq 0.05$) (Fig.6.5).

In mycelial systems of *P. velutina* extending from inoculum of 1 cm³ containing only one pre-colonised resource, phosphorus activity in wood resources colonised by *V.comedens* (R1), were significantly greater than in other treatments ($P \leq 0.05$) (Fig.6.6).

No significant difference was detected between treatments of the first and second resource for each inoculum size investigated (Fig.6.7). However, within treatment 5 (R1; *S.hirsutum*, R2; *V.comedens*) for mycelial systems extending from 1, 4 and

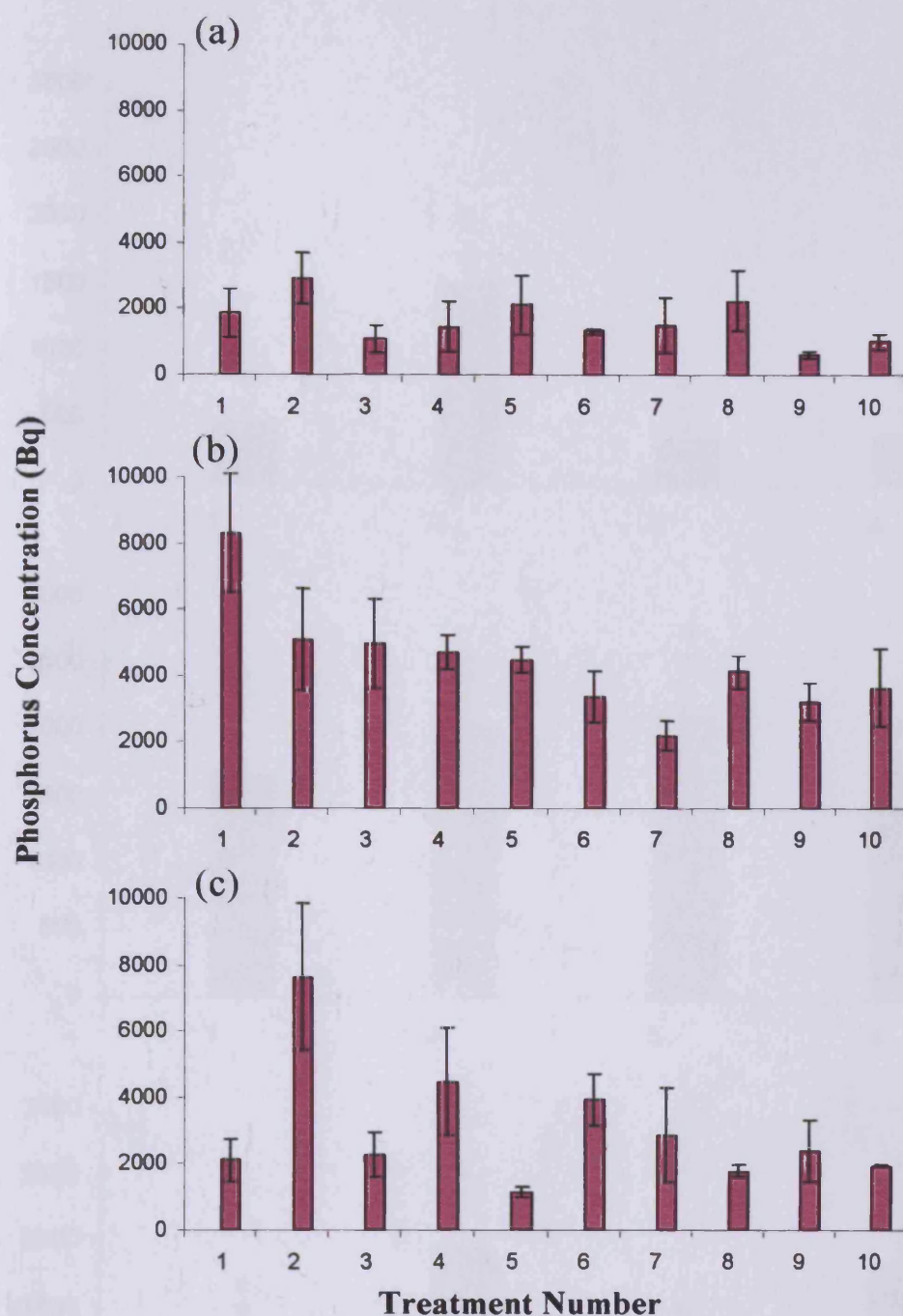


Figure 6.5 ^{32}P Concentration (Bq) in *P. velutina* inocula of 1 cm³(a), 4 cm³(b) and 16 cm³(c) for treatments 1-10 (see key below) No treatments were significantly different within inoculum group for each ($P > 0.05$). Error bars indicate standard error of the mean.

Treatment	Resource 1	Resource 2
1	<i>S. hirsutum</i>	
2	<i>V. comedens</i>	
3	<i>C. versicolor</i>	
4	uncolonised wood resource	
5	<i>S. hirsutum</i>	<i>V. comedens</i>
6	<i>C. versicolor</i>	<i>V. comedens</i>
7	<i>C. versicolor</i>	<i>S. hirsutum</i>
8	<i>S. hirsutum</i>	uncolonised wood resource
9	<i>V. comedens</i>	uncolonised wood resource
10	<i>C. versicolor</i>	uncolonised wood resource

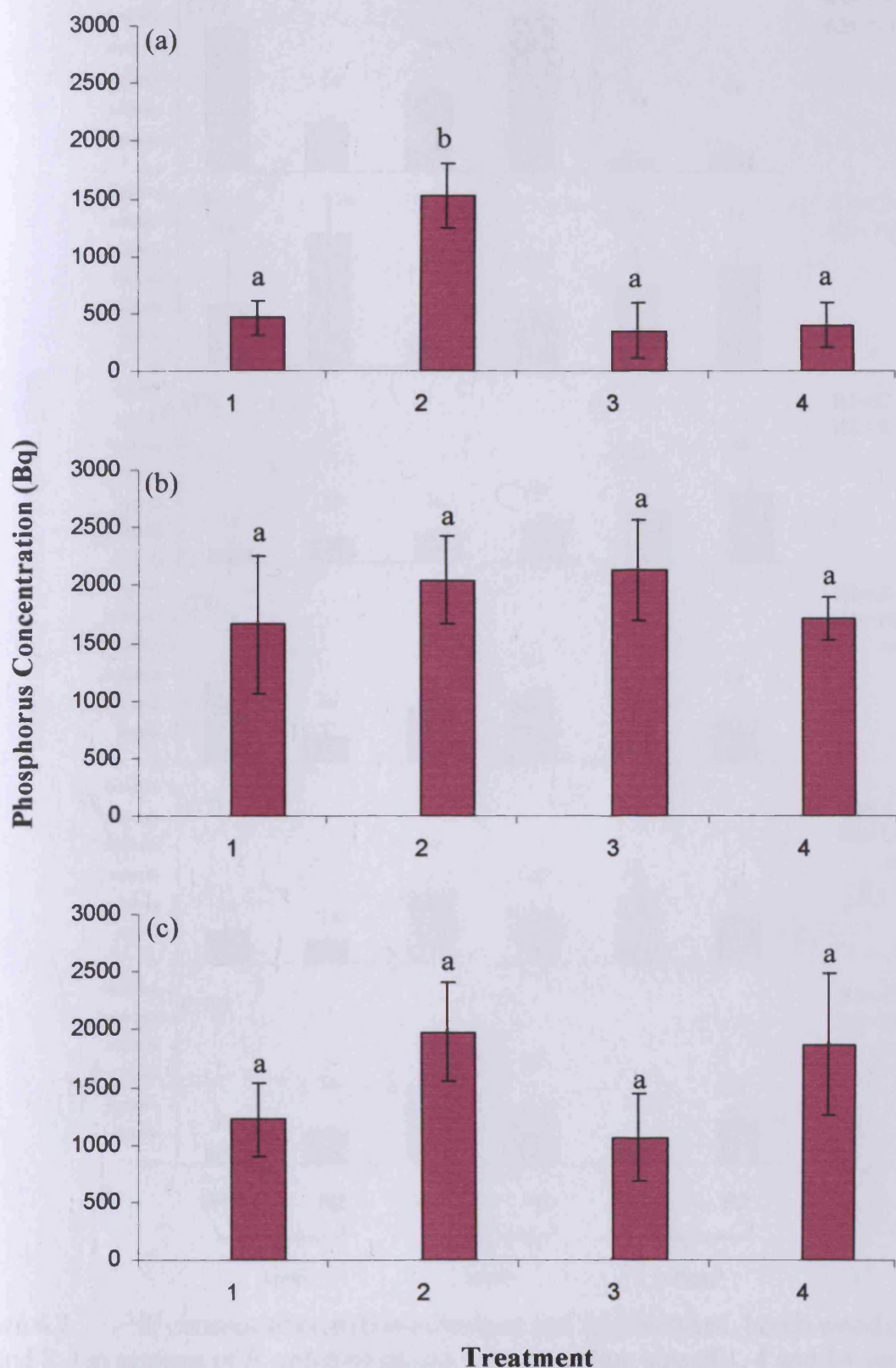


Figure 6.6 ^{32}P concentration (Bq) in wood resources (R1) pre-colonised by *Stereum hirsutum* (T1), *Vuilleminia comedens* (T2), *Coriolus versicolor* (T3). Fresh un-colonised resources (T4) in treatments encountering mycelium extending from 1 (a), 4 (b) and 16 cm³ (c) *P. velutina* inoculum. Bars with the same letter (a) within each inoculum size group are not significantly different (P > 0.05). Error bars indicate standard error of the mean.

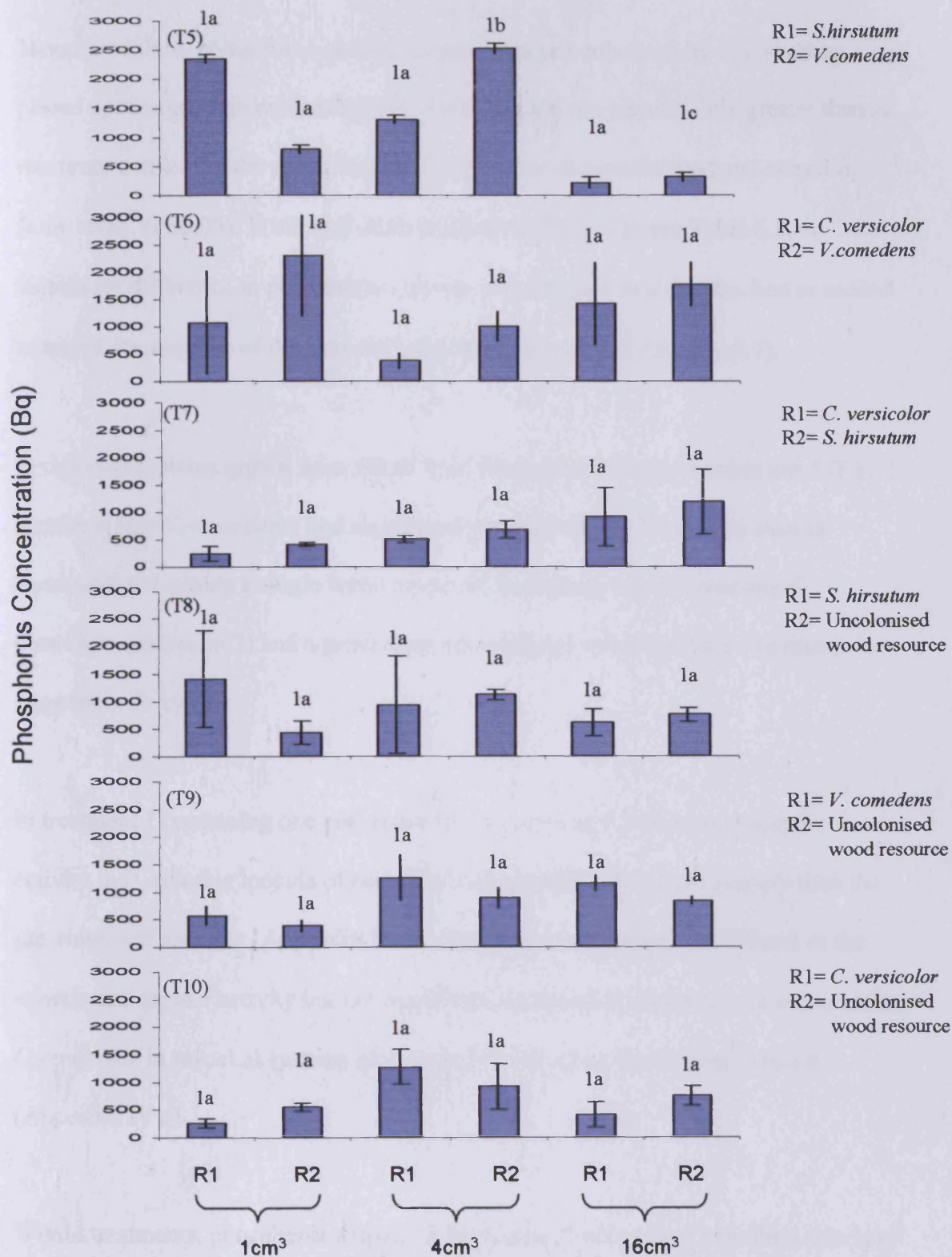


Figure 6.7 ^{32}P concentration in pre-colonised and un-colonised beech wood resources (R_1 and R_2) in systems of *P. velutina* grown from inoculum size of 1, 4 and 16 cm³ for treatments 5 - 10. R_1 and R_2 bars for all treatments with the same number (1) and treatments with the same letter (a) within each treatment between R_1 and R_2 are not significantly different ($P > 0.05$). Error bars indicate standard error of the mean.

16cm³ inoculum, phosphorus activity in resources pre-colonised by *V. comedens* placed upon mycelium extending from 4cm³ inocula was significantly greater than in resources containing the pre-coloniser *V. comedens* in mycelial systems extending from 16cm³ ($P \leq 0.05$). Within all other treatments, T6 to T10 see Table 6.2, no significant difference in phosphorus activity was detected in either the first or second resource, irrespective of the size of *P. velutina* inocula ($P \geq 0.05$) (Fig.6.7).

In mycelial systems grown from 16cm³ inoculum, resource one in treatment 5 (R1; *S. hirsutum*, R2; *V. comedens*) had significantly less ($P < 0.05$) ³²P activity than in treatments containing a single wood resource containing the pre-coloniser *V. comedens* (treatment 2) and a previously un-colonised wood resource (treatment 4). (Appendix IV c).

In treatment 1 containing one pre-colonised resource of *S. hirsutum*, phosphorus activity in *P. velutina* inocula of size 4cm³ had significantly higher activity than the pre-colonised resource (Appendix IV b). However in treatment 3, at the end of the experiment, greater activity but not significant remained in resources pre-colonised by *C. versicolor* in mycelial systems of *P. velutina* extending from 16cm³ inocula. (Appendix IV c).

Within treatments, phosphorus activity at harvest in *P. velutina* of inoculum size 1cm³ for treatment 10 (R1; *C. versicolor*, R2; previously un-colonised wood resource) was significantly greater than the activity remaining in the first resource pre-colonised by *C. versicolor* ($P \leq 0.05$) (Appendix IV a).

In mycelial systems extending from 4cm³, phosphorus activity was significantly greater in the first resources (R1) colonised by *S. hirsutum* in relation to treatment 5 (R1; *S. hirsutum*, R2; *V. comedens*) ($P \leq 0.05$) and in first resource ($P \leq 0.01$) of treatment 6, colonised by *C. versicolor*. (R1; *C. versicolor*, R2; *V. comedens*) (Appendix IV b).

Significantly less phosphorus activity remained ($P \leq 0.01$) in both the first and second resource in treatment 7 (R1; *C. versicolor*, R2; *S. hirsutum*) than activity remaining in *P. velutina* inocula of 4cm³. In treatment 9 (R1; *V. comedens*, R2, previously un-colonised resource), phosphorus activity was significantly less in the second previously un-colonised resource ($P \leq 0.05$) (Appendix IV b).

16cm³ inocula of *P. velutina* had significantly greater activity ($P \leq 0.01$) than both the first and second resource for treatments 5 (R1; *S. hirsutum*, R2; *V. comedens*), 10 (R1; *C.versicolor*, R2; un-colonised) and in treatment 8 (R1; *S. hirsutum*, R2; un-colonised) ($P \leq 0.05$). In treatment 6 (R1; *C.versicolor*, R2; *V. comedens*) phosphorus activity remaining was significantly greater in *P. velutina* inocula than in resources pre-colonised by *C. versicolor* ($P \leq 0.05$) (Appendix IV c).

6.3.4 Wood Decay Rates at Harvest for *P. velutina* and Resources R1 and R2

No significant differences were detected for treatments in wood decay rates of *P. velutina* inoculum of sizes 1, 4 and 16cm³ ($P \geq 0.05$) (Table 6.4).

Treatment	Treatment Pairings			Relative Wood Decay Rate (mg g ⁻¹ d ⁻¹)		
	R1	Pv	R2	R1	Pv	R2
(a)						
1	Sh	Pv		8.82 ± 0.79 ₁	4.36 ± 1.03 ₁	
2	Vc	Pv		11.21 ± 0.66 ₁	7.51 ± 0.20 ₁	
3	Cv	Pv		9.79 ± 0.27 ₁	6.51 ± 1.27 ₁	
4	Wb	Pv		7.69 ± 1.85 ₁	5.36 ± 2.42 ₁	
5	Sh	Pv	Vc	9.75 ± 0.63 ₁	7.55 ± 1.05 ₁	10.25 ± 0.31 ₁
6	Cv	Pv	Vc	10.82 ± 0.74 ₁	7.49 ± 0.80 ₁	9.80 ± 0.76 ₁
7	Cv	Pv	Sh	10.17 ± 1.10 ₁	5.28 ± 0.61 ₁	9.28 ± 1.52 ₁
8	Sh	Pv	Wb	9.75 ± 1.01 ₁	4.12 ± 1.60 ₁	5.36 ± 0.09 ₂
9	Vc	Pv	Wb	11.97 ± 2.30 ₁	7.20 ± 0.64 ₁	10.54 ± 0.16 ₁
10	Cv	Pv	Wb	10.93 ± 0.64 ₁	6.63 ± 0.34 ₁	9.45 ± 1.22 ₁
(b)						
1	Sh	Pv		8.77 ± 0.25 ₁	6.34 ± 1.46 ₁	
2	Vc	Pv		10.21 ± 0.14 ₁	5.22 ± 0.23 ₁	
3	Cv	Pv		11.52 ± 0.14 ₁	5.19 ± 0.49 ₁	
4	Wb	Pv		7.46 ± 2.46 ₁	8.51 ± 1.04 ₁	
5	Sh	Pv	Vc	10.86 ± 0.09 ₁	5.70 ± 0.22 ₁	11.80 ± 0.54 ₂
6	Cv	Pv	Vc	10.31 ± 0.49 ₁	5.57 ± 0.28 ₁	10.84 ± 0.49 ₃
7	Cv	Pv	Sh	9.83 ± 0.15 ₁	5.88 ± 0.40 ₁	9.13 ± 0.53 ₁
8	Sh	Pv	Wb	8.55 ± 0.32 ₁	5.69 ± 0.14 ₁	6.25 ± 0.42 ₁
9	Vc	Pv	Wb	5.92 ± 0.88 ₁	5.42 ± 0.36 ₁	8.08 ± 0.57 ₁
10	Cv	Pv	Wb	9.56 ± 0.62 ₁	7.29 ± 1.12 ₁	9.43 ± 0.30 ₁
(c)						
1	Sh	Pv		8.41 ± 0.86 ₁	4.42 ± 0.45 ₁	
2	Vc	Pv		10.38 ± 0.39 ₁	5.21 ± 0.03 ₁	
3	Cv	Pv		9.56 ± 1.44 ₁	4.13 ± 0.18 ₁	
4	Wb	Pv		7.53 ± 0.75 ₁	3.52 ± 0.19 ₁	
5	Sh	Pv	Vc	9.09 ± 0.93 ₁	4.45 ± 0.68 ₁	7.31 ± 0.39 ₁
6	Cv	Pv	Vc	9.58 ± 0.61 ₁	4.31 ± 0.32 ₁	8.96 ± 0.46 ₁
7	Cv	Pv	Sh	12.25 ± 0.24 ₂	4.43 ± 0.26 ₁	10.33 ± 0.27 ₂
8	Sh	Pv	Wb	7.8561 ± 0.37 ₁	4.12 ± 0.15 ₁	11.14 ± 1.33 ₁
9	Vc	Pv	Wb	8.68 ± 0.84 ₁	4.41 ± 0.38 ₁	9.29 ± 0.27 ₁
10	Cv	Pv	Wb	9.60 ± 1.05 ₁	4.80 ± 0.31 ₁	9.25 ± 0.43 ₁

Table 6.4 Decay rate data for *P. velutina* (Pv) inoculum 1 (a) 4 (b) and 16 cm³, where one or two resources (R1 & R2) were added behind the colony growing front. Decay data are represented with the standard error of the mean (n=5). Figures in the same column followed by the same letter are not significantly different (P>0.05) following one-way ANOVA and Priori tests.

There were no significant differences in wood decay rates in mycelial systems of *P. velutina* of inoculum size 1cm³. Treatments containing the pre-coloniser *S. hirsutum* (R1) and previously un-colonised resource (R2) (T8), had significantly less ($P \leq 0.05$) phosphorus activity in the previously un-colonised resource than the second resources within the remaining treatments. (Table 6.4a).

Significant differences in wood decay rates ($P \leq 0.05$) were indicated between the first resources in mycelial systems where *P. velutina* of inocula size was 4cm³. However, the data in this particular group were not normally distributed and variances were unequal therefore the indication of treatments that may be significantly different from each other were not detected, lowest wood decay rates were observed in treatment 9; resources pre-colonised by *V.comedens* with the value of $5.92 \pm 0.88 \text{ mg g}^{-1} \text{ d}^{-1}$ when an extra previously un-colonised wood resource was present (R1; *V.comedens*, R2; un-colonised) as opposed to systems containing only one resource pre-colonised by *V.comedens* where wood decay rates were $10.21 \pm 0.14 \text{ mg g}^{-1} \text{ d}^{-1}$ (Table 6.4b).

Wood decay rates of second resources contained within mycelial systems grown from 4cm³ inocula indicated highly significant differences ($P < 0.001$) as follows. Second resource pre-colonised by *V.comedens* relating to treatment 5 was significantly higher than the second resources containing the pre-colonised *S.hirsutum*, and previously un-colonised wood resource (T7, T8, & T9 respectively) (Table 6.4b). Second resource wood decay rates of previously un-colonised resources relating to treatment 9 were significantly lower ($P \leq 0.05$) than the second resource containing the pre-colonisers *V.comedens* (T5 & T6) and *S.hirsutum* (T7) (Table 6.4b). Similarly, second resources;

previously uncolonised (T9), were also were significantly lower ($P \leq 0.05$) than resources containing *V.comedens* (T5 & T6).

In mycelial systems extending from 16cm³ inocula, relative wood decay rates in first resources precolonised by *C. versicolor* paired against *S. hirsutum* (T7) were significantly higher ($P \leq 0.05$) than first resources of *S. hirsutum* (T8 R1; *S. hirsutum*, R2; uncolonised resource) and a fresh uncolonised resources (T4) (Table 6.4c). Wood decay rates of second resources containing the precoloniser *S. hirsutum* (T7, R1; *C. versicolor*, R2; *S. hirsutum*) were significantly greater ($P \leq 0.05$) that second resources precolonised by *V. comedens* when its first resource was also *S. hirsutum* (T5).

Wood decay rates within each treatment for mycelial systems containing a single resource (Treatments 1 –4) and systems containing two resource (Treatment 5 – 10), generally, *P. velutina* of inocula sizes 1, 4 and 16cm³ were lower than the pre-colonised / un-colonised resources $P < 0.05$, $P < 0.01$ (Table 6.4a, b & c).

6.3.5 Outcome of mycelial interactions in wood following a previous encounter by *P.velutina*

As in previous chapters 3, 4 and 5, *P. velutina* fully replaced mycelia contained within resources precolonised by *V.comedens* irrespective of the inoculum size *P. velutina* was extending from (Table 6.5-6.10).

In mycelial systems containing one resource, *P. velutina* of inoculum 1cm³, was able to fully replace 3 out of 5 resources precolonised by either *C.versicolor* or *S.hirsutum*

Treatment	Precolonised resource (R ₁)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
1	Sh	100	100	100	75	50
2	Vc	100	100	100	100	100
3	Cv	100	100	100	75	0
4	Wb	100	100	100	100	100
5	Sh	100	100	100	100	50
6	Cv	100	100	100	100	0
7	Cv	100	100	100	100	0
8	Sh	100	100	100	100	75
9	Vc	100	100	100	100	100
10	Cv	100	100	100	0	0

Table 6.5 Outcome of interactions between *P.velutina* of inoculum size 1 cm³, with pre-colonised resources (R₁) for treatments 1-10 (Table 6.2) of *Stereum hirsutum* and *Vuilleminia comedens* and *Coriolus versicolor* and colonisation by *P.velutina* of previously un-colonised wood resources.

Treatment	Precolonised / uncolonised resource (R ₂)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	Vc	100	100	100	100	100
6	Vc	100	100	100	100	100
7	Sh	100	100	100	100	75
8	Wb	100	100	100	100	100
9	Wb	100	100	100	100	100
10	Wb	100	100	100	100	100

Table 6.6 Outcome of interactions between *P.velutina* of inoculum size 1 cm³, with pre-colonised resources of *Stereum hirsutum* and *Vuilleminia comedens*, and colonisation by *P.velutina* of previously un-colonised wood resources, (R₂) for treatments 5-10 (Table 6.2).

Treatment	Precolonised resource (R ₁)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
1	Sh	100	100	75	50	50
2	Vc	100	100	100	100	100
3	Cv	100	100	75	75	25
4	Wb	100	100	100	100	100
5	Sh	100	100	100	100	50
6	Cv	100	100	100	100	100
7	Cv	100	100	100	100	100
8	Sh	100	100	100	50	50
9	Vc	100	100	100	100	100
10	Cv	100	100	100	100	50

Table 6.7 Outcome of interactions between *P.velutina* of inoculum size 4 cm³, with pre-colonised resources (R₁) for treatments 1-10 (Table 6.2) of *Stereum hirsutum* and *Vuilleminia comedens* and *Coriolus versicolor* and colonisation by *P.velutina* of previously un-colonised wood resources.

Treatment	Precolonised / uncolonised resource (R ₂)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	Vc	100	100	100	100	100
6	Vc	100	100	100	100	100
7	Sh	100	100	100	75	50
8	Wb	100	100	100	100	100
9	Wb	100	100	100	100	100
10	Wb	100	100	100	100	100

Table 6.8 Outcome of interactions between *P.velutina* of inoculum size 4 cm³, with pre-colonised resources of *Stereum hirsutum* and *Vuilleminia comedens*, and colonisation by *P.velutina* of previously un-colonised wood resources, (R₂) for treatments 5-10 (Table 6.2).

Treatment	Precolonised resource (R ₁)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
1	Sh	100	100	100	100	100
2	Vc	100	100	100	100	100
3	Cv	100	100	100	100	100
4	Wb	100	100	100	100	100
5	Sh	100	100	100	100	100
6	Cv	100	100	100	100	100
7	Cv	100	100	100	100	100
8	Sh	100	100	100	100	75
9	Vc	100	100	100	100	100
10	Cv	100	100	100	100	100

Table 6.9 Outcome of interactions between *P.velutina* of inoculum size 16 cm³, with pre-colonised resources (R₁) for treatments 1-10 (Table 6.2) of *Stereum hirsutum* and *Vuilleminia comedens* and *Coriolus versicolor* and colonisation by *P.velutina* of previously un-colonised wood resources.

Treatment	Precolonised / uncolonised resource (R ₂)	Percentage colonisation by <i>P.velutina</i> of pre-colonised/ uncolonised wood resources (%)				
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
5	Vc	100	100	100	100	100
6	Vc	100	100	100	100	100
7	Sh	100	100	100	100	100
8	Wb	100	100	100	100	100
9	Wb	100	100	100	100	100
10	Wb	100	100	100	100	100

Table 6.10 Outcome of interactions between *P.velutina* of inoculum size 16 cm³, with pre-colonised resources of *Stereum hirsutum* and *Vuilleminia comedens*, and colonisation by *P.velutina* of previously un-colonised wood resources, (R₂) for treatments 5-10 (Table 6.2).

(Table 6.5) the remaining two was partially replaced in respect to *S. hirsutum*, however, in relation to the precoloniser *C. versicolor*, in one resource, there was no apparent evidence that colonisation by *P. velutina* occurred (Table 6.5). This was also noted in second resources containing *C. versicolor* where one or two resources remained uncolonised by *P. velutina* (Table 6.5). In systems containing two resources, in relation to the first resource of treatment 8 (R1; *S. hirsutum*, R2; uncolonised resource) 1 out of 5 of the resources were partially replaced by *P. velutina* (Table 6.5). Similarly, 1 out of 5 of the second resources in relation to treatment 7 (R1; *C. versicolor* R2; *S. hirsutum*), *P. velutina* partially replaced *S. hirsutum* (Table 6.6).

A similar trend in mycelial systems extending from 4 cm³ inoculum was evident in terms of partial replacement by *P. velutina* of previously precolonised resources of *S. hirsutum* and *C. versicolor* where in systems containing either one or two resources, only 2 – 3 were fully replaced (Table 6.7 – 6.8). In treatment 10 (R1; *C. versicolor*, R2; uncolonised resource) full replacement by *P. velutina* occurred in 4 out of 5 of the resources previously containing *C. versicolor* (Table 6.7).

Mycelial systems of the largest inoculum size investigated (16cm³) fully replaced more of the replicates within each treatment when compared to smaller inoculum sizes 1 cm and 4 cm³. Only 1 resource, previously precolonised by *S. hirsutum* was partially replaced by *P. velutina* (Table 6.9), all other resource for mycelial systems containing one and two resources were fully replaced by *P. velutina*.

6.4 DISCUSSION

Phosphorus allocation/ translocation to wood resources has been shown be significantly affected by both the presence of other wood decay fungi in the wood resource (chapter 4) and the length in time of colonisation of wood resource by other wood decay fungi (chapter 5). This study has revealed that not only the above factors can have an influence on nutrient movement throughout mycelial systems, but also the size of inoculum of *P. velutina* can have an affect on the amount phosphorus uptake and translocation throughout mycelial systems.

During the experiment, lower activity was detected in systems extending from inocula of 1cm³. Dramatic responses exhibited by *P. velutina* appeared to occur in systems extending from 4 and 16 cm³ inoculum, were P activity between 12- 17 d (4cm³) and 6- 17 d (16cm³) after the addition of phosphorus was noticeably greater in systems containing only one resource. This indicated that phosphorus uptake by mycelia in the vicinity of *P. velutina* inoculum of 4 and 16 cm³ has the ability to transport greater phosphorus to wood resources of equal size or less when compared to 1 cm³ *P. velutina* inocula blocks. The difference in inoculum sizes on the ability to translocate phosphorus may be contributed to carbon limitation, where the greater the inoculum food base the higher ability for uptake and translocation of phosphorus to resources of different quality and decay states. This trend was also shown at harvest where concentration of ³²P remaining in both *P. velutina* inoculum and wood resources previously precolonised by other wood decay fungi or uncolonised, was considerably lower in treatments where *P. velutina* extended from 1cm³ inocula rather than *P. velutina* extending from the inoculum size of 4 cm or 16 cm³.

In mycelial systems containing a single resource, at harvest, in relation to 1 cm³ inoculum of *P. velutina*, activity remaining in resources precolonised by *V. comedens* was significantly greater than *S. hirsutum* and *C. versicolor*. This may be due to inoculum size affecting the combative ability displayed by *P. velutina*. Non nutrient studies have reported that size of inoculum does affect the combative behaviour exhibited by wood decay species, successful capture is often gained by increasing the mass ratio of one inoculum type to that of a resource containing another wood decay species (Holmer & Stenlid, 1993: Holmer & Stenlid 1997). In this study, all inoculum sizes were investigated, however, due to high variation within this data set, significant differences were not detected in relation to remaining P activity in precolonised resources within mycelial systems of *P. velutina* extending from 4 and 16cm³ inocula. High variation of ³²P activity of such resources and inoculum types investigated here has been reported in previous nutrient studies (Hughes 1993; Owens SL, 1997).

Phosphorus uptake and translocation is dependent on species, resource quality and inoculum / resource size (Wells, Hughes & Boddy (1990). Phosphorus activity in relation to the simultaneous arrival of two resources of different quality on mycelial systems of *P. velutina* was considerably greater in the second resources rather than the first. In this study, second resources were previously uncolonised fresh wood resources and resources precolonised by *V. comedens*, a poor combatant against *P. velutina*, past studies and this thesis, have demonstrated the preferential colonisation of the aforementioned resources when compared to an encounter of a resource already occupied by a more combative fungus such as *S. hirsutum*, *C. versicolor* (Boddy & Abdalla, 1998: Wells, Hughes & Boddy, 1990; Chapter 3: Chapter 4: Chapter 5).

Systems extending from 1 and 16cm³ inoculum had significantly affected the amount of phosphorus transported within treatments investigated. Precolonised resources of *V. comedens* contained within mycelial systems extending from 1 and 16cm³ inocula, significantly received more phosphorus when paired against *C. versicolor*. There was no significant differences detected in systems extending from inocula of 4 cm³, however, it was apparent that greater activity was observed in the second resources containing *V.comedens* and in an uncolonised resource when paired against *S. hirsutum* and *C.versicolor*. The latter two species are characteristically more antagonistic against *P.velutina* when compared against *V. comedens*.

Previous studies have reported that inoculum size does influence the outcome of interactions between wood decay fungi (Wells & Boddy, 2002; Holmer & Stenlid, 1993; Holmer & Stenlid 1997). This study has demonstrated that increasing the inoculum size has effectively increased the capability of capture of resources once occupied by other wood decay species, in particular, *C.versicolor* and *S. hirsutum*, where partial replacement by *P.velutina* occurred in wood resources precolonised by the aforementioned fungi contained within mycelial systems extending from 1 and 4 cm³. In mycelial systems extending from 16 cm³ all wood resources containing all test species investigated i.e. *C.versicolor*, *S.hirsutum* and *V.comedens* were fully replaced in exception of one resource, where partial replacement was evident of the resource containing the precoloniser *S.hirsutum*.

Overall, differences in inoculum size irrespective if a single or pair of wood resources of different resource quality are added to the mycelial system of *P. velutina*, generally has shown significant affects on phosphorus uptake and allocation in terms of combative responses between other wood decay species.

CHAPTER 7

SYNTHESIS

This investigation has used laboratory, microcosms of mycelial systems containing the basidiomycete *Phanerochaete velutina* on un-sterile soil in order to quantify the effect of the arrival (behind the mycelial foraging front) of colonised wood resources, differing in resource quality, decay state, and inoculum to resource ratio. The aim was to obtain a deeper understanding of the development of mycelial systems of *Phanerochaete velutina*, morphological responses, biomass reallocation, allocation and reallocation of nutrients (as ^{32}P) in response to the parameters tested.

In woodland ecosystems and in laboratory microcosms, *P. velutina*, a secondary coloniser, is a highly combative basidiomycete, where networks of mycelial cords ramifying at the soil/litter interface, interconnect with wood litter resources, which are nutrient units. Competition and foraging strategies are employed in order to gain occupancy of such wood litter resources, these resources are often already occupied by other wood decay fungi, for example, the secondary colonisers *Stereum hirsutum*, *Coriolus versicolor* and *Phlebia radiata* and the primary coloniser *Vuilleminia comedens*. In nature, wood litter components are discontinuously encountered in space and time, and competition for colonisation of resources that are of different quality, i.e. decay state, size, occurs (Wells & Boddy 2002). Types of encounter can have a major impact on the morphological responses exhibited by the forager, resulting in reallocation of mycelial biomass and nutrient translocation to that resource, or other resources already being colonised within the interconnecting mycelial network (Wells & Boddy, 1990; Boddy, 1993).

Previous studies in the laboratory and field have demonstrated that reallocation of mycelial biomass to newly encountered resources, placed ahead of the mycelial colony can occur (Dowson 1988, 1989; Wells & Boddy 1990, Boddy, 1999). The reallocation of mycelial biomass can vary in relation to where in the mycelial system an encounter of a new resource occurs, whether it is at the foraging front or indeed behind the colony margin of an established system (Wells, Harris & Boddy, 1998). Experiments reported in this thesis have demonstrated (by using quantifiable image analysis techniques) that mycelial systems of *P. velutina* containing resources differing in quality (i.e. resources that were pre-colonised by resource restricted fungi) placed behind the colony margin can cause dramatic changes in hyphal coverage and reallocation of mycelial biomass.

In previous studies of *P. velutina*, reallocation of mycelial biomass was only obvious when new resources encountered were larger than the original inoculum (Dowson, Rayner & Boddy 1986; Bolton, 1993). However reallocation within mycelial systems of *P. velutina* was detected in the present study in systems containing equal inoculum to new resource ratio (Chapter 3).

The affect of the addition of wood resources to mycelial systems was to cause polarisation of mycelial growth directed towards the resource encountered, has been demonstrated previously (Dowson et al 1998a; Hughes, 1993; Wells, Harris & Boddy, 1998a). Employing image analysis techniques to determine the mass fractal dimensions of mycelial systems to quantifying the extent to which mycelial systems permeate space in relation to the extent of the whole system was carried out in this thesis. As reported in previous studies an increase in mass fractal dimensions occurred

in mycelial systems of *P. velutina* in the sector containing a resource previously uncolonised (Wells, Harris & Boddy, 1998a), and shown for the first time here (Chapter 3) in a precolonised wood. Changes in mass fractal dimensions and the reallocation of mycelial biomass was affected by the addition of resources already colonised by other fungi, and these changes seem to reflect the ease / difficulty in which *P. velutina* is able to gain occupancy of the resources (Chapter 3). For example, the addition of a single resource either precolonised by *V. comedens*, *S. hirsutum*, *C. versicolor* or *P. radiata* to established mycelial systems of *P. velutina*, caused regression of non- connective mycelium far greater than in mycelial systems containing the resource precolonised by *V. comedens*. The morphology exhibited by *P. velutina* was not dissimilar in systems to treatments where a previously uncolonised fresh beech resource was added to the system. Reallocation of biomass in systems containing the *C. versicolor*, *S. hirsutum* and *P. radiata* was evident, hence the aforementioned fungi displayed more aggressive combative behaviour than the behaviour displayed by the poor combatant *V. comedens* (Chapter 3).

Wells, Hughes & Boddy (1990), stated that when mycelia encounter wood resource units already colonised by another wood decay fungus, placed ahead of the foraging front, phosphorus translocation can be altered to that resource. Similar results was found in previous studies conducted by Wells, Hughes & Boddy (1990), Wells & Boddy (1990) & Hughes & Boddy (1994), where bi-directional nutrient movement (both basipetal and acropetal nutrient movement) was observed non- destructively with time in mycelial systems of *P. velutina*. This data are in accordance with results demonstrated in this thesis (Chapter 4). Phosphorus uptake and translocation in established mycelial systems of *P. velutina* where two wood resources of equal

inoculum: resource ratio were placed behind the foraging mycelial front, differing in quality demonstrated by non – destructive monitoring of phosphorus activity, that ^{32}P was translocated to resources precolonised, then translocated back to the original inoculum and further beyond the inoculum to an additional uncolonised wood resource (Chapter 4).

The uniform addition of phosphorus solution to the soil in mycelial systems of *P. velutina* may contribute to the differences detected regarding biomass reallocation (Chapter 4). Regression in sectors containing the precolonised resources of *C. versicolor* and *P. radiata* were greater in systems where phosphorus solution was added uniformly to the soil and re-exploration of previously explored soil by *P. velutina* was observed in systems containing the precoloniser *V. comedens*. It appears that phosphorus translocation and uptake to wood resources by *P. velutina* is dependent on the precoloniser. Initially, less P activity was detected non-destructively in resources colonised by weaker competitors such as *V. comedens*. However more phosphorus was translocated to resources containing more aggressive wood decay fungi, presumably reflecting a greater demand for phosphorus translocation to resources containing more aggressive combatants in order for *P. velutina* to establish itself in the new resource.

Non- destructive monitoring of ^{32}P activity in resources of different quality of equal size has been demonstrated (chapter 4). Qualitative studies on the effect of decay state in relation to outcome of interactions between *P. velutina* and unit restricted fungi have been previously reported by Boddy & Abdalla (1998). In these studies it was evident that preferential colonisation of new resources differing in decay state

encountered by *P. velutina* were in favour of resources previously precolonised by other decay fungi for the much lesser colonisation time period of 6 weeks rather than the resources previously precolonised for two years. The addition of wood resources behind the foraging mycelial front that differ greatly in their decay state, (i.e. 8 week old and 3 year old previously precolonised resources containing either *V.comedens* or *S. hirsutum*) phosphorus translocation to such resources is dependent on resource type and colonisation age (Chapter 5).

The addition of one resource, either precolonised for 8 weeks or 3 years by the aforementioned unit restricted fungi demonstrated that initial phosphorus allocation was greater to the older precolonised resources of *V. comedens* and *S. hirsutum*, presumably reflecting a large demand for the transportation of nutrients at the early stages of combat by *P. velutina*. The opponents mycelium that has had longer time to colonise and occupy a new resource (i.e. *V. comedens* and *S. hirsutum*: 3 years) display a greater antagonistic defence to the intrusion of *P. velutina* when compared to the unit restricted fungi that have had less time to establish themselves in a new resource (Chapter 5). This trend was also apparent in mycelial systems containing two resources precolonised / uncolonised, where greatest ^{32}P activity was translocated initially to the older resources precolonised by *V. comedens* and *S. hirsutum*, however, an influx of phosphorus transported away from the older resources was translocated back to the original inoculum and onto the second resources containing a precolonised resource aged 8 weeks of either *V. comedens* or *S. hirsutum* thus further emphasising that phosphorus is allocated initially to resources that are demonstrate greater combative behaviour during early stages of combat, where phosphorus pathways are

reallocated elsewhere in the mycelial system to more favourable resources, such as the younger precolonised resources (Chapter 5).

Hughes and Boddy (1994), Hughes (1996), Wells Harris & Boddy (1998b) all have demonstrated that size of inoculum can significantly affect the amount of ^{32}P translocated within mycelial systems of *P. velutina*. This thesis has demonstrated that phosphorus uptake and translocation within mycelial systems appeared lower in systems extending from smaller inocula when compared to large inocula (Chapter 6). This trend was detected at harvest, where much lower activity remaining in *P. velutina* inoculum was apparent in systems extending from smaller inocula. More activity was apparent in systems of equal and larger inoculum to resource ratio. Overall, with time, resources containing either the precoloniser *V. comedens* and previously uncolonised resources were in preference of allocation of phosphorus by *P. velutina*.

Outcomes of interactions are dependent on resource quality, state of decay and inoculum to resource ratio (Boddy & Abdalla (1998); Wells & Boddy, (2002); Chapter 3, 5 & 6). This thesis has demonstrated that *P. velutina* can fully replace *V. comedens* in wood resource irrespective of the inoculum: resource ratio or state of decay (Chapter 3, 5 & 6). Mycelial systems of *P. velutina* extending from larger inoculum to resource ratio (4:1), showed a greater capability of fully replacing the fungi in wood resources previously precolonised by *C. versicolor* and *S. hirsutum*. Mycelial systems extending from a smaller inoculum to resource ratio (1:4) in some treatments containing resources precolonised by *C. versicolor* were still remaining at the end of the experiment and evidence of colonisation of that particular resource was evident by *P. velutina*. *C. versicolor* was able to withstand attack by mycelial systems

of *P. velutina* extending from smaller inoculum; resource ratio. Interestingly in mycelial systems with larger inoculum to resource ratios, *P. velutina* was able to fully replace *C. versicolor* (Chapter 6).

This concludes that the presence of unit restricted fungi, decay state, inoculum to resource ratio can significantly affect the outcomes of interactions (Chapter 3, 5 & 6), as well as the morphology and fractal dimensions in mycelial systems of *P. velutina* (Chapter 3). Phosphorus uptake and allocation within mycelial systems (Chapter 4, 5 and 6) was also changed. These findings are ecologically important in the life style of foraging fungi exhibited by *P. velutina*, where mycelial biomass and nutrients are reallocated within their foraging mycelial networks to favourable resources dependent on resource quality, where demand is greatest. Further work needs to be carried out in order to examine the effects of nutrient translocation not only between cord forming fungi and unit- restricted fungi, but also nutrient translocation between other interacting cord formers competing for resources already pre-colonised by unit restricted fungi. The size of the mycelial cord-forming colony may have a significant effect on the outcomes and nutrient translocation to the precolonised resource of *V. comedens*, *S. hirsutum*, *P. radiata* and *C. versicolor* investigated in this thesis.

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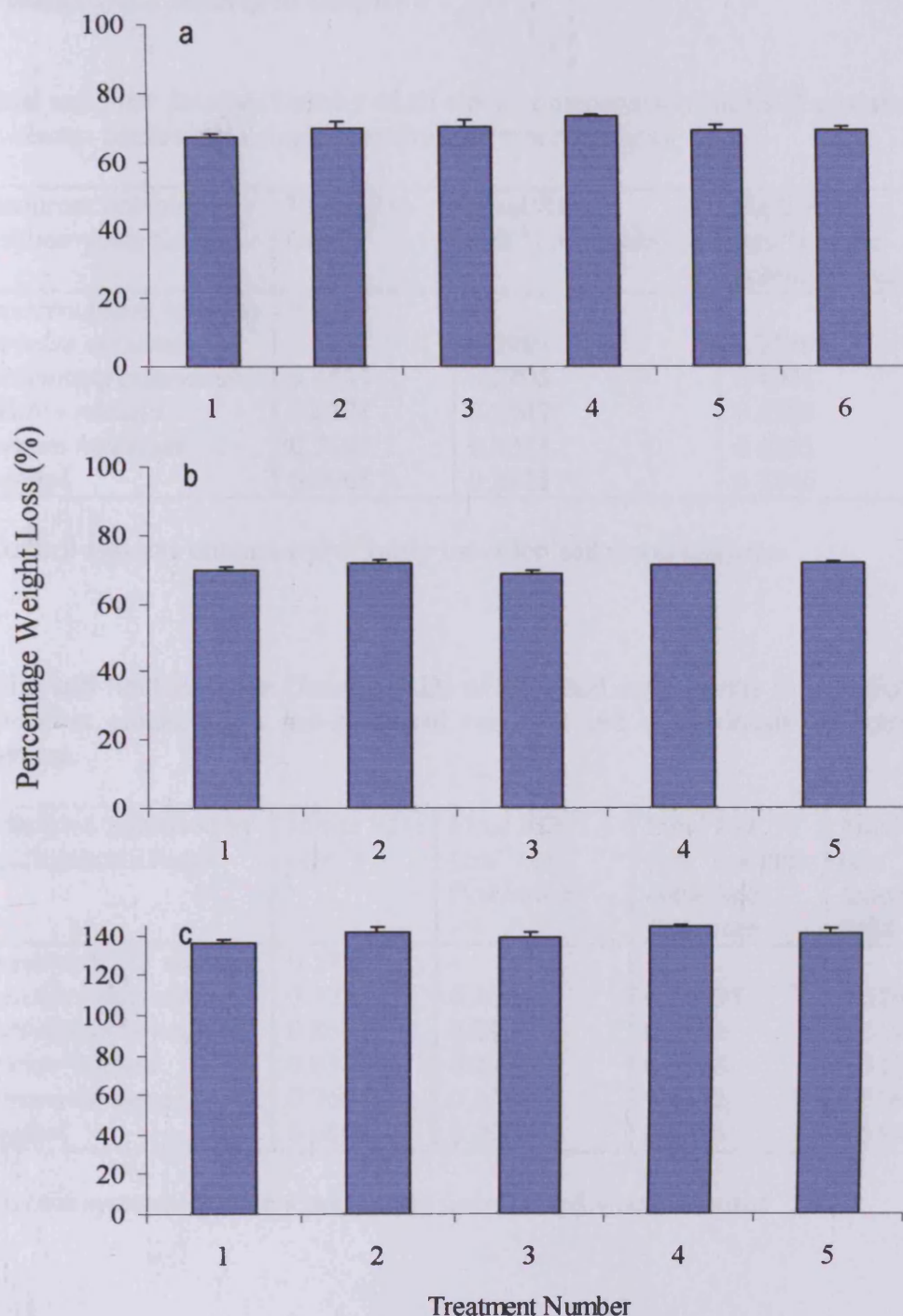
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Appendix I relating to chapter 3



Percentage weight loss of wood resources colonised by *Phanerochaete velutina* (a), pre-colonised resources (b). Total percentage weight loss in mycelial systems was recorded (c). Treatments 1-5 represent mycelial systems of *P. velutina* interacting with *Coriolus versicolor*, *Vuilleminia comedens*, *Phlebia radiata*, *Stereum hirsutum* respectively. Treatments 5 represent positive control systems where *P. velutina* systems contained a previously un-colonised resource and treatment 6 represents negative controls where pre-colonised resources were absent in mycelial cord systems.

Appendix II (a) relating to Chapter 4

Initial and final Relative Density of all wood components in mycelial systems of *P. velutina* containing a single precolonised wood resource

Resources colonised by basidiomycete fungi	Initial RD (cm ⁻³)	Final RD (cm ⁻³) of <i>P. velutina</i>	Final RD (cm ⁻³) of pre-colonised resources
<i>Phanerochaete velutina</i>	0.578	-	-
<i>Coriolus versicolor</i>	0.7235	0.2989	0.3549
<i>Vuilleminia comedens</i>	0.8631	0.2796	0.4071
<i>Phlebia radiata</i>	0.8371	0.2948	0.4388
<i>Stereum hirsutum</i>	0.7697	0.3023	0.4535
*control	0.6068	0.2938	0.3846

*Control systems contain a previously un-colonised wood resource

Initial and final Relative Density (RD) of all wood components in mycelial systems of *P. velutina* containing a pre-colonised resource and a previously un-colonised wood resource

Resources colonised by basidiomycete fungi	Initial RD (cm ⁻³)	Final RD (cm ⁻³) of <i>P. velutina</i>	Final RD (cm ⁻³) of pre-colonised resources	Final RD (cm ⁻³) of second wood baits
<i>Phanerochaete velutina</i>	0.578	-	-	-
<i>Coriolus versicolor</i>	0.7235	0.3004	0.34495	0.52603
<i>Vuilleminia comedens</i>	0.8631	0.2936	0.3951	0.51915
<i>Phlebia radiata</i>	0.8371	0.2971	0.3188	0.5109
<i>Stereum hirsutum</i>	0.7697	0.3154	0.4312	0.5160
*control	0.6068	0.2968	0.3605	0.5530

*Control systems contain a previously uncolonised wood resource

Appendix II (b) relating to Chapter 4

Mean percentage allocation of translocated ^{32}P in *P. velutina* inoculum wood blocks, wood pre-colonised by other decay fungi and previously uncolonised wood blocks 82 days after addition to soil near the inoculum or the pre-colonised resource.

Percentage allocation of translocated ^{32}P

Group	Treatment Number	Wood Resource type	<i>P. velutina</i> Inoculum	Pre-colonised inoculum	Previously un-colonised resource
1	1	<i>C. versicolor</i>	74.85 _{1a}	25.15 _{1b}	
	2	<i>C. versicolor</i>	38.16 _{1a}	61.84 _{1b}	
	3	<i>C. versicolor</i>	36.14 _{1a}	25.41 _{1a}	38.46 _{1a}
	4	<i>C. versicolor</i>	37.18 _{1a}	40.77 _{1a}	22.05 _{1a}
2	1	<i>V. comedens</i>	40.83 _{1a}	59.17 _{1a}	
	2	<i>V. comedens</i>	42.46 _{1a}	57.55 _{1a}	
	3	<i>V. comedens</i>	38.05 _{1a}	22.62 _{1a}	39.33 _{1a}
	4	<i>V. comedens</i>	18.02 _{1a}	49.60 _{1a}	32.38 _{1a}
3	1	<i>P. radiata</i>	66.74 _{1a}	33.26 _{1b}	
	2	<i>P. radiata</i>	50.49 _{12a}	49.51 _{1a}	
	3	<i>P. radiata</i>	40.29 _{12a}	39.18 _{1a}	20.53 _{1a}
	4	<i>P. radiata</i>	21.55 _{2a}	51.46 _{1a}	27.00 _{1a}
4	1	<i>S. hirsutum</i>	49.42 _{1a}	50.58 _{1a}	
	2	<i>S. hirsutum</i>	47.84 _{1a}	52.16 _{1a}	
	3	<i>S. hirsutum</i>	30.55 _{1a}	38.81 _{1a}	30.64 _{1a}
	4	<i>S. hirsutum</i>	30.01 _{1a}	28.76 _{1a}	41.23 _{1a}
5	1	Un-colonised	30.73 _{1a}	69.27 _{2a}	
	2	Un-colonised	36.17 _{1a}	63.83 _{12a}	
	3	Un-colonised	25.95 _{1ab}	23.33 _{12a}	50.72 _{1b}
	4	Un-colonised	36.50 _{1a}	24.87 _{1a}	38.62 _{1a}

Within each group, data in the same column followed by the same number, and in the same row followed by the same letter, are not significantly different ($P < 0.05$)

Appendix III (a) relating to Chapter 5

Treatment	<i>P. velutina</i> paired against;		R ₁	I	R ₂	Total Uptake (R ₁ + I + R ₂) (Bq)
	R ₁	R ₂				
1	Sh 3yrs	-	1959.07 ± 655 _{1a}	6345.91 ± 974 _{1b}	-	8304.98 ₁
2	Sh 8wks	-	1656.45 ± 597 _{1a}	8313.95 ± 1785 _{1b}	-	9970.4 ₁
3	Vc 3yrs	-	3228.13 ± 151 _{1a}	6530.20 ± 529 _{1b}	-	9758.33 ₁
4	Vc 8wks	-	2041.28 ± 382 _{1a}	5103.15 ± 1549 _{1a}	-	7144.43 ₁
5	Sh 3yrs	Vc 8wks	348.38 ± 125 _{1,2a}	1864.47 ± 607 _{1a}	798.20 ± 317 _{1a}	3011.05 ₁
6	Sh 3yrs	Sh 8wks	1163.82 ± 413 _{1a}	2817.1 ± 814 _{1a}	1678.16 ± 657 _{1a}	5659.08 ₁
7	Vc 3yrs	Vc 8wks	833.96 ± 230 _{1b}	2384.7 ± 503 _{1a}	1090.80 ± 152 _{1a,b}	4309.46 ₁
8	Vc 3yrs	Sh 8wks	933.08 ± 403 _{1a}	2509.16 ± 586 _{1a}	1349.65 ± 422 _{1a}	4791.89 ₁
9	Vc 3yrs	Wb	2513.4 ± 1506 _{1a}	7150.04 ± 3938 _{1a}	2094.78 ± 1094 _{1a}	11758.22 ₁
10	Sh 3yrs	Wb	1245.2 ± 654 _{1,3a}	2859.57 ± 1257 _{1a}	1126.39 ± 455 _{1a}	5231.16 ₁
11	Vc 8wks	Wb	934.24 ± 891 _{1a}	4124.73 ± 481 _{1a}	1125.42 ± 91 _{1a}	6184.39 ₁
12	Sh 8wks	Wb	1309.06 ± 297 _{1a,b}	2865.58 ± 531 _{1a}	1072.62 ± 225 _{1b}	5247.26 ₁

Mean concentration of ³²P (Bq) in *P. velutina inoculum* (I) wood blocks and wood resources precolonised by *Vuilleminia comedens* (Vc) and *Stereum hirsutum* (Sh) for either 3 years or 8 weeks (R₁) and resources precolonised or uncolonised by *Vuilleminia comedens* (Vc) and *Stereum hirsutum* (Sh) for 8 weeks (R₂). Data in the same column followed by the same number, and in the same row followed by the same letter, are not significantly different (P<0.05).

Appendix III (b) relating to Chapter 5

Treatment	<i>P.velutina</i> paired against;		R ₁	I	R ₂
	R ₁	R ₂			
1	Sh _{3yrs}	-	23.5891	76.4109	
2	Sh _{8wks}	-	16.61368	83.38632	
3	Vc _{3yrs}	-	33.08076	66.91924	
4	Vc _{8wks}	-	28.57163	71.42837	
5	Sh _{3yrs}	Vc _{8wks}	11.57005	61.92092	26.50903
6	Sh _{3yrs}	Sh _{8wks}	20.56553	49.78018	29.65429
7	Vc _{3yrs}	Vc _{8wks}	19.35184	55.3364	25.31176
8	Vc _{3yrs}	Sh _{8wks}	19.47207	52.36264	28.1653
9	Vc _{3yrs}	Wb	21.37568	60.80886	17.81545
10	Sh _{3yrs}	Wb	23.80352	54.66417	21.53232
11	Vc _{8wks}	Wb	15.10642	66.69583	18.19775
12	Sh _{8wks}	Wb	24.9475	54.61098	20.44153

Mean percentage allocation of translocated ³²P (Bq) by *P.velutina inoculum* (I) wood blocks to wood resources precolonised by *Vuilleminia comedens* (Vc) and *Stereum hirsutum* (Sh) for either 3 years or 8 weeks (R₁) and resources precolonised or uncolonised by *Vuilleminia comedens* (Vc) and *Stereum hirsutum* (Sh) for 8 weeks (R₂).

Appendix IV (a) relating to Chapter 6; 1 cm³ inocula

Treatment	<i>P.velutina</i> paired against;		R ₁	I	R ₂	Total Uptake (R ₁ + I + R ₂) (Bq)
	R ₁	R ₂				
1	Sh	-	460.58 ± 144.236 _{1a}	1871.22 ± 717.963 _{1a}	-	2331.80
2	Vc	-	1522.61 ± 284.374 _{1a}	2893.63 ± 789.63 _{1a}	-	4416.24
3	Cv	-	346.77 ± 243.203 _{1a}	1091.31 ± 416.277 _{1a}	-	1438.08
4	Wb	-	393.12 ± 382 _{1a}	1461.18 ± 757.97 _{1a}	-	1854.30
5	Sh	Vc	2350.31 ± 350.081 _{1a}	2121.45 ± 869.53 _{1a}	788.58 ± 270.01 _{1a}	5260.34
6	Cv	Vc	1072.27 ± 939.565 _{1a}	1329.93 ± 85.153 _{1a}	2287.67 ± 1102.5 _{1a}	4689.87
7	Cv	Sh	231.67 ± 134.475 _{1a}	1509.97 ± 840.415 _{1a}	409.77 ± 29.55 _{1a}	2151.41
8	Sh	Wb	1405.56 ± 877.059 _{1a}	2235.58 ± 912.547 _{1a}	424.43 ± 216.31 _{1a}	4065.57
9	Vc	Wb	578.08 ± 166.578 _{1a}	640.97 ± 101.08 _{1a}	383.19 ± 101.33 _{1a}	1602.24
10	Cv	Wb	259.78 ± 79.808 _{1a}	1013.75 ± 213.433 _{1b}	550.84 ± 76.15 _{1a}	1824.37

Mean concentration of ³²P (Bq) in *P.velutina inoculum* (I) 1cm³ wood blocks and wood resources (4cm³) un-colonised (Wb) or pre-colonised for 8 weeks by *Stereum hirsutum* (Sh), *Vuilleminia comedens* (Vc) and *Coriolus versicolor* (R₁ / R₂) Data in the same column followed by the same number, and in the same row followed by the same letter, are not significantly different (P<0.05).

Appendix IV (b) relating to Chapter 6; 4 cm³ inocula

Treatment	<i>P.velutina</i> paired against;		R ₁	I	R ₂	Total Uptake (R ₁ + I + R ₂) (Bq)
	R ₁	R ₂				
1	Sh	-	1656.45 ± 596.707 _{1a}	8313.95 ± 1784.63 _{1b}	-	9970.43
2	Vc	-	2041.28 ± 382.272 _{1a}	5103.15 ± 1549.29 _{1a}	-	7144.43
3	Cv	-	2129.82 ± 433.472 _{1a}	4998.37 ± 1345.81 _{1a}	-	7128.19
4	Wb	-	1705.88 ± 189.291 _{1a}	4705.04 ± 510.42 _{1a}	-	6410.92
5	Sh	Vc	1303.69 ± 568.165 _{1b}	4469.96 ± 393.07 _{1a}	2527.2 ± 843.984 _{1a}	8300.85
6	Cv	Vc	368.52 ± 141.444 _{1b}	3367.25 ± 785.16 _{1a}	1000.2 ± 263.42 _{1a}	4735.97
7	Cv	Sh	505.91 ± 69.633 _{1a}	2201.48 ± 462.14 _{1b}	680.46 ± 147.67 _{1a}	3387.85
8	Sh	Wb	934.24 ± 891.425 _{1a}	4124.73 ± 480.57 _{1a}	1125.42 ± 90.62 _{1a}	6184.39
9	Vc	Wb	1263.58 ± 415.074 _{1b}	3205.19 ± 577.47 _{1a}	888.94 ± 184.073 _{1b}	5357.71
10	Cc	Wb	1278.95 ± 316.327 _{1a}	3639.36 ± 1156.16 _{1a}	924.36 ± 415.965 _{1a}	5842.67

Mean concentration of ³²P (Bq) in *P.velutina inoculum* (I) 4cm³ wood blocks and wood resources (4cm³) un-colonised (Wb) or pre-colonised for 8 weeks by *Stereum hirsutum* (Sh), *Vuilleminia comedens* (Vc) and *Coriolus versicolor* (R₁ / R₂) Data in the same column followed by the same number, and in the same row followed by the same letter, are not significantly different (P<0.05).

Appendix IV (c) relating to Chapter 6; 16 cm³ inocula

Treatment	<i>P. velutina</i> paired against;		R ₁	I	R ₂	Total Uptake (R ₁ + I + R ₂) (Bq)
	R ₁	R ₂				
1	Sh	-	1656.45 ± 596.707 _{1a}	2099.14 ± 652 _{1a}	-	3755.59
2	Vc	-	2041.28 ± 382.272 _{1a}	7625.78 ± 2207.49 _{1a}	-	9667.06
3	Cv	-	2129.82 ± 433.472 _{1a}	2273.39 ± 686.9 _{1a}	-	4403.21
4	Wb	-	1705.88 ± 189.291 _{1a}	4472.32 ± 1621.97 _{1a}	-	6178.20
5	Sh	Vc	222.94 ± 41.751 _{2a}	1160.45 ± 155.3 _{1b}	319.34 ± 107.452 _{1a}	1702.73
6	Cv	Vc	1416.36 ± 757.654 _{1a}	3943.31 ± 757.26 _{1b}	1769.29 ± 398.41 _{1a}	7128.96
7	Cv	Sh	909.73 ± 535.312 _{1a}	2863.64 ± 1427.98 _{1a}	1186.64 ± 596.007 _{1a}	4960.01
8	Sh	Wb	607.61 ± 246.063 _{1a}	1775.81 ± 187.47 _{1b}	755.93 ± 130.044 _{1a}	3139.34
9	Vc	Wb	1154.79 ± 148.797 _{1a}	2360.4 ± 934.19 _{1a}	762.04 ± 71.662 _{1a}	4277.23
10	Cc	Wb	404.94 ± 225.161 _{1a}	1928.62 ± 27.11 _{1b}	747.87 ± 177.518 _{1a}	3081.43

Mean concentration of ³²P (Bq) in *P. velutina* inoculum (I) 16cm³ wood blocks and wood resources (4cm³) un-colonised (Wb) or pre-colonised for 8 weeks by *Stereum hirsutum* (Sh), *Vuilleminia comedens* (Vc) and *Coriolus versicolor* (R₁ / R₂) Data in the same column followed by the same number, and in the same row followed by the same letter, are not significantly different (P<0.05).

